

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF CUMENE
(CAS NO. 98-82-8)
IN F344/N RATS AND B6C3F1 MICE
(INHALATION STUDIES)



NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

February 2009

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National Institutes of Health
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

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SUMMARY

Background

Cumene occurs naturally in petroleum and is used as a solvent, in gasoline and diesel fuels, and as the principal chemical in the production of phenol and acetone. We studied cumene to determine if it caused cancer in rats or mice.

Methods

We exposed groups of 50 male and female rats and mice to air containing cumene 6 hours per day for 2 years. Rats and male mice were exposed to concentrations of 250, 500, or 1,000 parts per million (ppm) of cumene in air, and female mice were exposed to concentrations of 125, 250, or 500 ppm. Similar groups of 50 animals were exposed to clean air in the same inhalation chambers 6 hours per day as the untreated control groups. Tissues from more than 40 sites were examined for every animal.

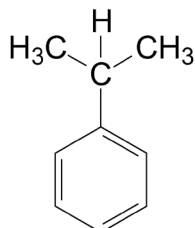
Results

All groups of animals exposed to cumene exhibited hyperplasia of the epithelial tissues of the nose, and exposed male and female mice experienced metaplasia and hyperplasia of the lung. Male mice also had nonneoplastic lesions in the forestomach and liver. Adenomas of the respiratory epithelium of the nose were observed in male and female rats, and male rats had increased incidences of renal tubule adenoma or carcinoma (combined) and interstitial cell adenoma of the testis. Adenomas and carcinomas of the lung were markedly increased in male and female mice exposed to cumene. The rate of liver neoplasms was also increased in exposed female mice, and a few hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland were seen in male mice exposed to the highest concentration of cumene.

Conclusions

We conclude that the increased occurrences of adenomas of the epithelium of the nose in male and female rats, of renal tubule adenoma or carcinoma (combined), of adenomas and carcinomas of the lung in male and female mice, and of liver neoplasms in female mice were caused by exposure to cumene. The occurrence of interstitial cell adenoma of the testis in male rats and hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland in male mice may also have been associated with exposure to cumene.

ABSTRACT



CUMENE

CAS No. 98-82-8

Chemical Formula: C_9H_{12} Molecular Weight: 120.19

Synonyms: Cumol; isopropylbenzene; isopropylbenzol; (1-methyl/ethyl)benzene; 2-phenylpropane

Cumene is produced in a modified Friedel-Crafts reaction process that uses acidic catalysts to alkylate benzene with propylene. Cumene is the principal chemical used in the production of phenol and acetone. Cumene is used to produce acetophenone, α -methylstyrene, diisopropylbenzene, and dicumylperoxide; as a thinner; as a constituent of some petroleum-based solvents; in gasoline blending, diesel fuel, and high-octane aviation fuel; and as a raw material for peroxides and oxidation catalysts. Because cumene is a good solvent for fats and resins, it has been suggested as a replacement for benzene in many industrial applications. Cumene occurs naturally in petroleum and in a variety of foodstuffs. Cumene was nominated for study by the NIEHS because of its high production volume, presence in gasoline and other fuels, potential for human exposure, and lack of existing carcinogenicity test data. Male and female F344/N rats and B6C3F1 mice were exposed to cumene (greater than 99.9% pure) by inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat bone marrow, and mouse peripheral blood.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were exposed to cumene vapor at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 days. All rats exposed to 4,000 ppm died on day 1, and two male and three female rats exposed to 2,000 ppm died by day 4. Mean body weights of 2,000 ppm rats were significantly less than those of the chamber controls. Rats exposed to 2,000 ppm that died early were severely lethargic following daily exposure. Liver and kidney weights of all exposed groups were increased. Accumulation of minimal to mild hyaline droplets was observed in the renal tubular cortex of males exposed to concentrations of 250 to 2,000 ppm.

2-WEEK STUDY IN MICE

Groups of five male and five female mice were exposed to cumene vapor at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm, 6 hours plus T_{90} (12 minutes) per

day, 5 days per week for 17 days. All mice exposed to 4,000 ppm died on day 1; all mice exposed to 2,000 ppm died on day 2, and four female mice exposed to 1,000 ppm died by day 4. Mean body weights of all exposed groups were similar to those of the chamber controls. Mice exposed to 2,000 ppm were severely lethargic after the first exposure. The four female mice exposed to 1,000 ppm that died early exhibited signs of lethargy and ataxia. Liver weights, both relative and absolute, were increased in all groups of surviving males and in the 250 and 500 ppm female groups.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to cumene vapor at concentrations of 0, 62.5, 125, 250, 500, or 1,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. All rats survived to the end of the study, and mean body weights of all exposed groups were similar to those of the chamber controls. Kidney and liver weights of 250 ppm or greater males and liver weights of 1,000 ppm females were significantly greater than those of the chamber controls. There were significant differences between exposed and chamber control females in the relative length of time spent in the estrous stages. The amount of α_2 -globulin in the right kidneys was significantly increased in male rats exposed to 125 ppm or greater. The incidences of medullary granular casts in males exposed to 250 ppm or greater were significantly increased. The severities of renal tubule cortex hyaline droplet accumulation and regeneration increased with increasing exposure concentration in male rats.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice were exposed to cumene vapor at concentrations of 0, 62.5, 125, 250, 500, or 1,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Eight 1,000 ppm females died during week 1 of the study. Mean body weights of males exposed to 500 or 1,000 ppm were significantly less than those of the chamber controls. The eight 1,000 ppm female mice that died during the first week of the study exhibited clinical signs of acute toxicity, including lethargy or ataxia. Liver weights of

mice exposed to 500 or 1,000 ppm were significantly increased. The weight of the cauda epididymis and the spermatid count were significantly decreased in 1,000 ppm males.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were exposed to cumene vapor at concentrations of 0, 250, 500, or 1,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival of all exposed groups of rats was similar to that of the chamber controls. Mean body weights of 1,000 ppm females were slightly less than those of the chamber controls during the second year of the study but were similar to the chamber controls at the end of the study.

Incidences of adenoma of the respiratory epithelium in the nose occurred with a positive trend in males and were significantly increased in all exposed groups of males and in 250 ppm females. Incidences of hyperplasia of basal cells in the olfactory epithelium in the nose of all exposed groups and hyperplasia of the respiratory epithelium in the nose of all exposed groups of males and 1,000 ppm females were significantly increased.

The incidences of renal tubule adenoma in all exposed groups of males, renal tubule carcinoma in 500 and 1,000 ppm males, and renal tubule adenoma or carcinoma (combined) in all exposed groups of males were increased; the difference from chamber controls for the combined incidence was significant at 500 ppm. The incidences of hyperplasia of the renal tubule and transitional epithelium of the renal pelvis in 500 and 1,000 ppm males and mineralization of the renal papilla in all exposed groups of males were significantly greater than those of the chamber controls.

The incidence of interstitial cell adenoma (including bilateral) of the testis was significantly increased in 1,000 ppm male rats, and there was a positive trend in the incidences across all groups.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were exposed to cumene vapor at concentrations of 0, 125 (female mice only), 250, 500, or 1,000 (male mice only) ppm,

6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. An exposure concentration-related decrease in survival occurred in male mice, and the survival of 1,000 ppm males was significantly less than that of the chamber controls. Mean body weights of 1,000 ppm males were generally less than those of the chamber controls after week 8 of the study, and those of 500 ppm females were less from week 28 until week 76 of the study.

The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) in all exposed groups of mice occurred with positive trends and were significantly greater than those in the chamber controls. The incidences of alveolar epithelial bronchiole metaplasia and bronchiole hyperplasia were significantly increased in all exposed groups of mice. *p53* and *K-ras* mutations were found in 52% and 87% of lung neoplasms in exposed mice compared to 0% and 14% in the chamber controls, respectively.

In female mice, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) occurred with positive trends and were significantly increased in the 500 ppm group. In male mice, there were significant increases in the incidences of eosinophilic foci of the liver.

The incidences of hemangiosarcoma in the spleen and of follicular cell adenoma in the thyroid gland were significantly increased in 1,000 ppm male mice.

In the nose, the incidences of olfactory epithelium atrophy, basal cell hyperplasia of the olfactory epithelium, atypical hyperplasia of the olfactory epithelium, hyperplasia of olfactory epithelium glands, and suppurative inflammation were generally significantly increased in 500 and 1,000 ppm males and 500 ppm females. The incidence of squamous metaplasia of the respiratory epithelium was significantly increased in 500 ppm females. The incidence of basal cell hyperplasia was also significantly increased in 250 ppm females.

The incidences of epithelial hyperplasia of the forestomach in the 500 and 1,000 ppm groups of males and the incidences of ulceration and inflammation of the

forestomach in 1,000 ppm males were significantly increased.

GENETIC TOXICOLOGY

Cumene was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535, when tested with and without liver S9 activation enzymes. Cumene induced small, but significant, increases in micronucleated polychromatic erythrocytes in bone marrow of male rats treated by intraperitoneal injection. In contrast, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation for 3 months.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma (combined). Increased incidences of interstitial cell adenoma of the testis may have been related to exposure to cumene. There was *some evidence of carcinogenic activity* of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose. There was *clear evidence of carcinogenic activity* of cumene in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. The increased incidences of hemangiosarcoma in the spleen and follicular cell adenoma in the thyroid gland in male mice may have been related to cumene exposure. There was *clear evidence of carcinogenic activity* of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Increased incidences of hepatocellular adenoma or carcinoma (combined) in female mice were also considered to be related to exposure to cumene.

Exposure of male rats to cumene resulted in nonneoplastic lesions of the kidney characteristic of $\alpha 2u$ -globulin accumulation. Exposure to cumene resulted in nonneoplastic lesions in the nose of male and female rats; the lung, nose, liver, and forestomach of male mice; and the lung and nose of female mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Report Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Cumene

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in air	0, 250, 500, or 1,000 ppm	0, 250, 500, or 1,000 ppm	0, 250, 500, or 1,000 ppm	0, 125, 250, or 500 ppm
Body weights	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	1,000 ppm males less than the chamber control group	Exposed groups similar to the chamber control group
Survival rates	26/50, 23/50, 27/50, 24/50	21/50, 27/50, 31/50, 32/50	38/50, 34/50, 30/50, 23/50	37/50, 36/50, 39/50, 35/50
Nonneoplastic effects	<p><u>Nose</u>: olfactory epithelium, hyperplasia, basal cell (0/50, 19/50, 27/49, 26/50); respiratory epithelium, hyperplasia (0/50, 15/50, 16/49, 23/50)</p> <p><u>Kidney</u>: renal tubule, hyperplasia (0/50, 3/50, 8/50, 6/50); papilla, mineralization (5/50, 35/50, 44/50, 41/50); pelvis, transitional epithelium, hyperplasia (3/50, 5/50, 14/50, 15/50)</p>	<p><u>Nose</u>: olfactory epithelium, hyperplasia, basal cell (0/50, 14/48, 25/50, 31/50); respiratory epithelium, hyperplasia (0/50, 0/48, 4/50, 6/50)</p>	<p><u>Lung</u>: alveolar epithelium, bronchiole, metaplasia (5/50, 43/50, 42/50, 39/50); bronchiole, hyperplasia (0/50, 11/50, 17/50, 18/50)</p> <p><u>Nose</u>: olfactory epithelium, atrophy (4/50, 13/50, 11/49, 38/48); olfactory epithelium, hyperplasia, basal cell (0/50, 0/50, 15/49, 33/48); olfactory epithelium, hyperplasia, atypical (0/50, 0/50, 5/49, 11/48); olfactory epithelium, glands, hyperplasia (3/50, 11/50, 9/49, 23/48); inflammation, suppurative (2/50, 2/50, 9/49, 6/48)</p> <p><u>Liver</u>: eosinophilic focus (6/50, 5/50, 16/50, 14/50)</p> <p><u>Forestomach</u>: epithelium, hyperplasia (2/50, 7/50, 8/50, 13/49); ulcer (1/50, 4/50, 6/50, 6/49); inflammation (0/50, 2/50, 1/50, 5/49)</p>	<p><u>Lung</u>: alveolar epithelium, bronchiole, metaplasia (0/50, 42/50, 49/50, 47/50); bronchiole, hyperplasia (0/50, 17/50, 10/50, 14/50)</p> <p><u>Nose</u>: olfactory epithelium, atrophy (4/50, 11/50, 9/50, 18/50); olfactory epithelium, hyperplasia, basal cell (0/50, 1/50, 11/50, 25/50); olfactory epithelium, hyperplasia, atypical (0/50, 0/50, 2/50, 10/50); olfactory epithelium, glands, hyperplasia (1/50, 4/50, 4/50, 11/50); respiratory epithelium, metaplasia, squamous (0/50, 0/50, 1/50, 6/50); inflammation, suppurative (0/50, 1/50, 3/50, 7/50)</p>

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Cumene

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Neoplastic effects	<u>Nose:</u> respiratory epithelium, adenoma (0/50, 7/50, 18/49, 10/50) <u>Kidney:</u> renal tubule, adenoma or carcinoma (2/50, 5/50, 8/50, 7/50)	<u>Nose:</u> respiratory epithelium, adenoma (0/50, 5/48, 4/50, 3/50)	<u>Lung:</u> alveolar/bronchiolar adenoma (13/50, 31/50, 31/50, 29/50); alveolar/bronchiolar carcinoma (9/50, 19/50, 32/50, 33/50); alveolar/bronchiolar adenoma or carcinoma (19/50, 38/50, 42/50, 43/50)	<u>Lung:</u> alveolar/bronchiolar adenoma (1/50, 26/50, 36/50, 38/50); alveolar/bronchiolar carcinoma (3/50, 16/50, 20/50, 34/50); alveolar/bronchiolar adenoma or carcinoma (4/50, 31/50, 42/50, 46/50) <u>Liver:</u> hepatocellular adenoma (18/50, 23/50, 27/50, 29/50); hepatocellular adenoma or carcinoma (25/50, 26/50, 29/50, 36/50)
Equivocal findings	<u>Testes:</u> interstitial cell, adenoma (18/50, 14/50, 13/50, 9/50); bilateral, interstitial cell, adenoma (18/50, 24/50, 27/50, 37/50); interstitial cell and bilateral interstitial cell adenoma (36/50, 38/50, 40/50, 46/50)		<u>Spleen:</u> hemangiosarcoma (0/50, 0/50, 0/49, 4/50) <u>Thyroid gland:</u> follicular cell, adenoma (0/50, 0/50, 0/49, 3/50)	
Level of evidence of carcinogenic activity	Clear evidence	Some evidence	Clear evidence	Clear evidence
Genetic toxicology				
<i>Salmonella typhimurium</i> gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535, with and without S9			
Micronucleated erythrocytes				
Rat bone marrow <i>in vivo</i> :	Positive			
Mouse peripheral blood <i>in vivo</i> :	Negative			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence and some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on cumene on May 16, 2007, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On May 16, 2007, the draft Technical Report on the toxicology and carcinogenicity study of cumene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of cumene by describing the uses and human exposure possibilities of the chemical, the genetic toxicity profile, the study design, and the survival, body weights, and neoplastic and nonneoplastic lesions observed in the 3-month and 2-year studies. Dr. D.E. Malarkey, NIEHS, described the histopathologic details of the lesions seen in the respiratory tract and some of the supplemental molecular pathology findings. The proposed conclusions were *clear evidence of carcinogenic activity* of cumene in male F344/N rats, *some evidence of carcinogenic activity* of cumene in female F344/N rats, and *clear evidence of carcinogenic activity* of cumene in male and female B6C3F1 mice.

Dr. Mirsalis, the first principal reviewer, had no major scientific criticisms. He felt unconvinced that the rat micronucleus data showed a truly positive response.

Dr. Walker, the second principal reviewer, agreed that the micronucleus response was at best equivocal. He felt the study was well performed and especially liked the inclusion of *K-ras* and *p53* mutation analyses for the lung neoplasms.

Dr. Pino, the third principal reviewer, inquired about a statistically significant increase in interstitial cell adenomas of the testes in male rats that was not included in

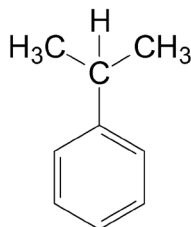
the results section of the report. He asked about the reason for the distinction between clear and some evidence for the nasal adenomas in male and female rats.

Dr. Crump noted two other sites in male mice for which significant occurrences of tumors were not included in the results or conclusions: hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland. He also queried whether the kidney tumors in male rats merited a classification of clear evidence.

Dr. Kerkvliet said that a written response had been submitted by the American Chemistry Council and that an oral presentation would be given by Dr. George Kruzam. Dr. Kruzam noted that in the introduction of the report cumene was incorrectly identified as being used in styrene synthesis. He suggested that the abstract of the report include a statement about the relevance of the findings to human disease and include genotoxicity results from the literature.

In consideration of the conclusion statements, Dr. Walker suggested that a statement be added indicating the association of the nonneoplastic kidney lesions in male rats with α_2 u-globulin. Dr. Crump moved that mention be added of the three additional lesions: testicular adenoma in male rats and hemangioma of the spleen and thyroid gland follicular cell adenomas in male mice. Dr. J.R. Bucher, NIEHS, said that if these tumors were considered equivalent to equivocal evidence, they would be termed "may have been related" to chemical administration. The panel voted unanimously with seven votes to approve the inclusion of these three lesions as "may have been related" plus a statement concerning the α_2 u-globulin accumulation.

INTRODUCTION



CUMENE

CAS No. 98-82-8

Chemical Formula: C_9H_{12} Molecular Weight: 120.19

Synonyms: Cumol; isopropylbenzene; isopropylbenzol; (1-methyl/ethyl)benzene; 2-phenylpropane

CHEMICAL AND PHYSICAL PROPERTIES

Cumene is a volatile, colorless liquid with a sharp, penetrating aromatic or gas-like odor (*Merck*, 1989; Cavender, 1994; NIOSH, 2005). Cumene has a boiling point of $152.4^{\circ}C$ (Lide, 1995), a melting point of $-96.0^{\circ}C$ (Lide, 1995), and a vapor pressure of 3.2 mm Hg at $20^{\circ}C$ (Verschuere, 1983). Cumene is insoluble in water but is miscible with acetone, benzene, and ethanol (Lide, 1995). Cumene forms cumene hydroperoxide upon prolonged exposure to the air (NIOSH, 2005).

PRODUCTION, USE, AND HUMAN EXPOSURE

Cumene as a pure chemical intermediate is produced in a modified Friedel-Crafts reaction process that uses acidic catalysts to alkylate benzene with propylene. The majority of cumene is manufactured with a solid phosphoric acid catalyst; the remainder is made with an aluminum chloride catalyst (Schulz *et al.*, 1993). The annual production of cumene in the United States was 4.49 billion pounds in 1993 and 5.63 billion pounds in 1995 (Anonymous, 1995; Kirschner, 1996; HSDB, 2003).

Cumene is the principal chemical used in the production of phenol and acetone via the chemical intermediate cumene hydroperoxide (the hydroperoxide is cleaved to phenol and acetone in an acidic environment). Cumene is used to produce acetophenone, α -methylstyrene, diisopropylbenzene, and dicumylperoxide. Cumene can also be used as a thinner for paints, enamels, and lacquers; as a constituent of some petroleum-based solvents such as naphtha; in gasoline blending, diesel fuel, and high-octane aviation fuel; and as a raw material for peroxides and oxidation catalysts such as polymerization catalysts for acrylic and polyester-type resins. It is also a good solvent for fats and resins and, as such, has been suggested as a replacement for benzene in many industrial applications (Parmeggiani, 1983; Verschuere, 1983; Mannsville, 1985; *Merck*, 1989; ACGIH, 1993; Anonymous, 1993; *Hawley's*, 1993; Schulz *et al.*, 1993; HSDB, 2003).

Cumene occurs naturally in petroleum crudes and coal tar (Verschuere, 1983). It also occurs in a variety of natural substances, including essential oils from plants, marsh grasses, and a variety of foodstuffs. Trace quantities have been detected in papaya, sapodilla fruit, and Australian honey. Cumene has been detected but not quantified in fried chicken, tomatoes, Concord grapes,

cooked rice, oat groats, baked potatoes, Beaufort cheese, fried bacon, dried legumes (beans, split peas, lentils), southern pea seeds, and Zinfandel wine (HSDB, 2003).

The potential for worker exposure exists during production and processing from petroleum refining. General population exposure to cumene results from inhalation of air contaminated with cumene from evaporation of petroleum products and from cigarette smoke; additional exposure may result from ingestion of food (HSDB, 2003). Cumene is released into the environment as a result of cumene production, processing, and transport, petroleum refining and the evaporation and combustion of petroleum products, the transportation and distribution of motor fuel, and the use of a variety of products containing cumene. Cumene has been detected in air samples from Los Angeles, CA, at concentrations as high as 144 $\mu\text{g}/\text{m}^3$ and in groundwater, surface water, and drinking water (Jackson, 1985; USEPA, 1997; HSDB, 2003).

The threshold-limit value-time-weighted average recommended by the American Conference of Governmental Industrial Hygienists (2005) for cumene is 50 ppm (245 mg/m^3); a short-term exposure limit (STEL) has not been determined. The Occupational Safety and Health Administration permissible exposure limit is 50 ppm, with a skin designation, averaged over an 8-hour work shift; a STEL has not been determined (29 CFR, Part 1910.1000). The exposure limit recommended by the National Institute for Occupational Safety and Health for cumene is 50 ppm, with a skin notation, averaged over a 10-hour work shift (NIOSH, 2005). The United States Environmental Protection Agency (1997) has assigned cumene to carcinogen category D (not classifiable or inadequate human or animal data) and determined an inhalation reference concentration of 0.4 mg/m^3 using uncertainty factors to reflect a daily exposure concentration without appreciable risk of deleterious effects during a lifetime.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Metabolism, disposition, and pharmacokinetic studies of cumene in rats following oral, intravenous injection, or nose-only inhalation administration have demonstrated that the chemical is well absorbed. Following absorption, a small quantity (approximately 5%) of the cumene was exhaled unchanged, but the major portion was

oxidized at the benzylic carbon to 2-phenyl-2-propanol (Figure 1), with subsequent oxidation to 2-hydroxy-2-phenylpropanoic acid, 2-phenyl-1,2-propanediol, and phenyllactic acid. A minor pathway is oxidation of a methyl group to 2-phenyl-1-propanol and subsequently to 2-phenylpropanoic acid and 2-phenylmalonic acid. A minor metabolite may be phenylmalonic acid (Senczuk and Litewka, 1976; NRC, 1981; Parmeggiani, 1983; Gosselin *et al.*, 1984; Lee, 1987; Sato and Nakajima, 1987; Slaughter and Jeffcoat, 1989, 1990, 1992; Ishida and Matsumoto, 1992; Cavender, 1994; USEPA, 1997). These metabolites were excreted mainly in the urine in conjugated form. Oxidation occurs in both hepatic and extrahepatic tissues, including the lung (Sato and Nakajima, 1987). Cumene does not bioaccumulate in tissues (ACGIH, 1993). There is no known biotransformation product that would suggest toxicity.

TOXICITY

Experimental Animals

Table 1 lists LC_{50} and LD_{50} values for cumene in rats, mice, and rabbits.

Mice exposed to cumene vapor exhibited dilation of cutaneous blood vessels, grades of central nervous system depression, narcosis, depression of respiration, and death, depending on the concentration and duration of exposure. The narcosis was characterized by slow induction and long duration relative to benzene and toluene.

When rats were dosed by repeated gastric intubation with 154, 462, or 769 mg cumene/kg body weight for 194 days, no changes in body weights, hematology, or histopathology of the liver or kidney were found, but at 462 and 769 mg/kg , increases in kidney weights were observed (ACGIH, 1993). Subcutaneous administration of 1 mL/kg daily for 2 weeks did not lower the femoral bone marrow cell population of rats (ACGIH, 1993).

In an inhalation study, exposure of rabbits to 1,323 ppm (6,496 mg/m^3) cumene for up to 180 days resulted in no changes in behavior or body weight gain (Fabre *et al.*, 1955). In the same study, inhalation exposure of rats to 509 ppm (2,499 mg/m^3) for 180 days produced a decrease in body weight gain limited to the initial part of the study and congestion of the lung, liver, spleen, kidney, and adrenal gland; higher exposure concentrations [814 ppm (3,997 mg/m^3) and 1,323 ppm (6,496 mg/m^3)]

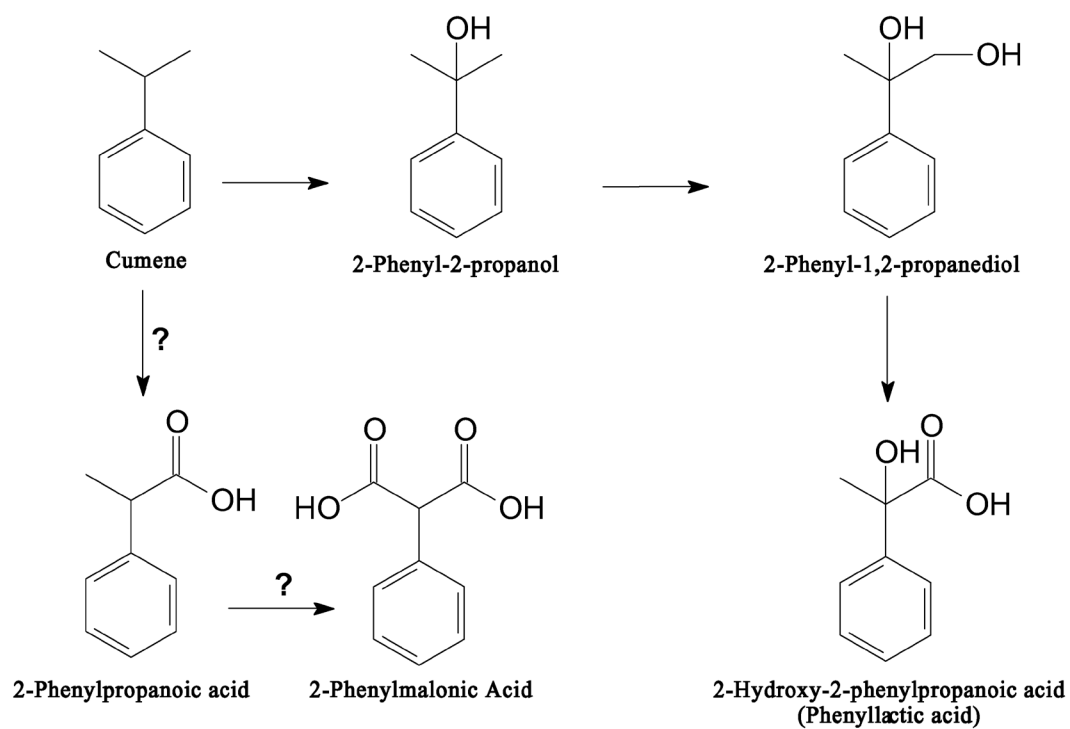


FIGURE 1
Known Metabolites of Cumene

TABLE 1
Acute Toxicity Values for Cumene^a

Species	Route of Administration	LC ₅₀ /LD ₅₀
Rats	Inhalation (4-hour)	8,000 ppm
Rats	Oral	1.4 g/kg (male)
Mice	Inhalation (7-hour)	2,000 ppm
Mice	Oral	12.75 g/kg
Rabbits	Dermal	12.3 mL/kg

^a Cavender, 1994; Cushman *et al.*, 1995

killed the rats within 16 hours of exposure. Daily inhalation exposure of rats to 500 ppm for 5 months resulted in no significant changes in the peripheral blood; however, hyperemia and congestion were noted in the lung, liver, and kidney of exposed animals (ACGIH, 1993). Jenkins *et al.* (1970) used inhalation techniques to expose rats, guinea pigs, dogs, and monkeys to 244 ppm (1,195 mg/m³) for 8 hours per day, 5 days per week for 30 exposures or to 3.7 ppm (18 mg/m³) or 30 ppm (146 mg/m³) continuously for 90 to 130 days and reported essentially negative findings.

Cushman *et al.* (1995) exposed groups of 21 male and 21 female Fischer 344 rats to cumene vapor at concentrations of 0, 100, 500, or 1,200 ppm for 6 hours per day, 5 days per week for 13 weeks. A subsequent 13-week study with a 4-week recovery period was conducted in groups of 15 male and 15 female rats at exposure concentrations of 0, 50, 100, 500, or 1,200 ppm. No exposure-related changes in body weights, mortality, a functional observation battery, auditory brain stem responses, brain measurements, or nervous system histopathology were observed. Motor activity decreases seen only in 500 and 1,200 ppm males in the first study were not replicated in the second study. The 500 and/or 1,200 ppm groups showed transient decreases in body weight gain and feed consumption, increases in water consumption, and changes in several hematologic and clinical chemistry parameters. No exposure-related ophthalmologic findings or effects on spermatogenesis occurred. Liver, kidney, and adrenal gland weights were increased in the 500 and 1,200 ppm groups. Renal proximal tubule cell hypertrophy, hyperplasia, and hyaline droplet formation were observed in 500 and 1,200 ppm males.

Daily inhalation exposure to 500 ppm cumene for 150 days was reported to induce hyperemia of the lung, liver, and kidney (species and sex not specified), but no changes occurred in the peripheral blood or bone marrow of animals exposed to 1,300 to 1,400 ppm for 180 days (Parmeggiani, 1983).

Humans

Cumene is an eye, skin, and mucous membrane irritant (ACGIH, 1993; HSDB, 2003; NIOSH, 2005). Short-term exposure to cumene may cause dizziness, headache, drowsiness, slight incoordination, and unconsciousness (HSDB, 2003). Prolonged contact with liquid cumene may cause erythema or blisters (NIOSH/OSHA, 1981; Gosselin *et al.*, 1984).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Timed-pregnant Sprague-Dawley rats (25 per group) were exposed to cumene vapor for 6 hours per day on gestational days 6 through 15 at target concentrations up to 1,200 ppm (Neeper-Bradley, 1989a). In this study, maternal toxicity was evidenced at 1,200 ppm by significant reductions in body weight gain and treatment-related clinical signs of toxicity (perioral wetness and encrustation), decreased feed consumption, and increased relative liver weight; gestational parameters and fetal body weights per litter were unaffected by the exposure. In a subsequent study, this investigator found that pregnant rabbits exposed to 2,300 ppm cumene vapor during organogenesis evidenced consistent maternal toxicity (reductions in weight gain and feed consumption and increased relative liver weight).

(Neeper-Bradley, 1989b). Gestational parameters such as the number of corpora lutea; the total, nonviable, or viable implantations per litter; sex ratio; pre- or postimplantation loss; fetal body weights per litter; and malformations exhibited no significant changes.

Humans

No studies of reproductive or developmental effects of cumene in humans were found in a review of the literature.

CARCINOGENICITY

No reports of carcinogenic effects of cumene in experimental animals or evidence of carcinogenic effects in humans in epidemiology studies or case reports were found in the literature. However, the NTP has conducted carcinogenicity studies of ethylbenzene (a structurally related chemical) administered by inhalation and reported induction of renal tubule neoplasms in male and female F344/N rats, testicular adenoma in male F344/N rats, alveolar/bronchiolar neoplasms in male B6C3F1 mice, and hepatocellular neoplasms in female B6C3F1 mice (NTP, 1999). In contrast, ethylbenzene was not carcinogenic in male or female CD (Sprague-Dawley) rats gavaged daily with 500 mg/kg, 4 to 5 days per week for 104 weeks (Maltoni *et al.*, 1985).

GENETIC TOXICITY

There are few published reports on the mutagenicity of cumene, and the limited information available suggests that cumene is not mutagenic in standard assays. Cumene was reported to be nonmutagenic in *Salmonella typhimurium* tester strains with and without metabolic activation (Simmon *et al.*, 1977; Florin *et al.*, 1980),

and results obtained with cumene in several industry-sponsored *in vitro* and *in vivo* genetic toxicity studies in mammalian cell test systems also indicated no potential for mutagenic or clastogenic activity (GLSC, 1985a,b; Curren, 1987; Putman, 1987; Yang, 1987).

As noted earlier, cumene is structurally related to ethylbenzene. NTP (1999) studies have demonstrated that ethylbenzene is not mutagenic in *S. typhimurium* tester strains with or without S9 activation enzymes (Zeiger *et al.*, 1992), but treatment of cultured mouse lymphoma L5178Y cells with ethylbenzene in the absence of S9 resulted in significantly increased mutation frequencies at the tk⁺/ locus (McGregor *et al.*, 1988). No induction of sister chromatid exchanges or chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with ethylbenzene with or without S9 enzymes (NTP, 1999), and no increases in the frequencies of micronucleated erythrocytes were seen in peripheral blood of male or female B6C3F1 mice exposed to ethylbenzene by inhalation for 3 months (Witt *et al.*, 2000).

STUDY RATIONALE

Cumene was nominated for study by the NIEHS because of its high production volume, presence as a component in gasoline and other fuels, potential for human exposure, and lack of existing carcinogenicity test data. Inhalation was chosen for these studies because this is the primary route of human exposure. Cumene was studied for toxic and carcinogenic effects in rats and mice exposed by whole body inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat bone marrow, and mouse peripheral blood.

MATERIALS AND METHODS

PROCUREMENT

AND CHARACTERIZATION OF CUMENE

Cumene was obtained from Sunoco, Inc. (Philadelphia, PA), in one lot (200556852) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the study laboratory at Battelle Toxicology Northwest (Richland, WA), by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO), and by Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO), (Appendix I). Reports on analyses performed in support of the cumene studies are on file at the National Institute of Environmental Health Sciences.

Lot 200556852 of the chemical, a colorless liquid with a sharp, penetrating, aromatic odor, was identified as cumene by the analytical chemistry laboratory and Chemir/Polytech Laboratories, Inc., using ^1H -nuclear magnetic resonance spectroscopy, gas chromatography/mass spectrometry, and/or infrared spectroscopy. For lot 200556852, Karl Fischer titration indicated a water content ranging from approximately 50 to 220 ppm; elemental analyses for carbon and hydrogen were in agreement with the theoretical values for cumene. Gas chromatography by one system detected no impurities greater than 0.05%, and the purity was determined to be approximately 100%. Using gas chromatography by another system, the area percent purity for the major cumene peak was 99.9%, and no peaks were detected with an area percent greater than 0.1%. The overall purity of lot 200556852 was determined to be greater than 99.9%.

To ensure stability, the bulk chemical was stored at controlled room temperature in the original shipping containers (55-gallon metal drums). Stability was monitored by the study laboratory during the 2-week, 3-month, and 2-year studies with gas chromatography. No degradation of the bulk chemical was detected.

VAPOR GENERATION

AND EXPOSURE SYSTEM

The design of the vapor generation and exposure system was influenced by the relatively high boiling point of cumene (approximately 152°C) and the need to reach relatively high concentrations. Therefore, with the exception of individual chamber inlets, all vapor transport lines and dilution air were heated to the minimum temperature needed to move vapor to the chambers without condensation. A bulk supply of cumene was held in an 8-gallon stainless steel chemical reservoir and pumped through a preheater into the top of a heated glass column filled with glass beads to increase the surface area for evaporation. Heated nitrogen entering the column from below vaporized the chemical as it conveyed it out of the generator. Generator output was controlled by the delivery rate of the chemical metering pump.

Cumene vapor was transported to the exposure room through transport lines at an elevated temperature to prevent condensation. In the distribution manifold cabinet, the vapor was mixed with additional heated air before it entered a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution air flow rate, all of which were monitored by the exposure operator. The pressure in the distribution manifold was fixed to ensure constant flow through the manifold and into the chambers as the flow of vapor to each chamber was adjusted.

Electronically actuated metering valves controlled the flow to each chamber. In addition, an exposure-shutoff valve, mounted in series with each chamber-metering valve, controlled vapor delivery to each chamber. Vapor was diverted to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. To start the exposure, the valves were

opened to allow the flow of vapor to reach the chamber-metering valves and move into individual temperature-controlled delivery lines to each chamber. The vapor was then injected into the chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m³. A condensation particle counter (Model 3022A, TSI, Inc., St. Paul, MN) was used to count the particles in all chambers before and during generation to ensure that cumene vapor, and not aerosol, was produced. No particle counts greater than 200 particles/cm³ were detected.

VAPOR CONCENTRATION MONITORING

Concentrations of cumene in the exposure chambers were monitored by an on-line gas chromatograph. Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 26 (2-year studies) minutes during each 6-hour exposure period using Hasteloy-C stream-select and gas-sampling valves (Valco Instruments Co., Houston, TX) in a separate, heated valve oven. The sample lines composing each sample loop were made from Teflon[®] tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow. Summaries of the chamber vapor concentrations are given in Appendix I, Tables I2 through I4.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of cumene in nitrogen supplied by a permeation tube standard generator (Kin-Tek Model 491, Kin-Tek Laboratories, Inc., La Marque, TX). The on-line gas chromatograph was calibrated prior to the start of each study, three times during the 2-week studies, and monthly during the 3-month and 2-year studies by a comparison of chamber concentration data to data from grab samples that were collected with charcoal sam-

pling tubes (ORBO[™]-101, Supelco, Bellefonte, PA), extracted with toluene containing 1,2,4-trimethylbenzene as an internal standard, and analyzed by an off-line gas chromatograph. The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standard solutions of cumene and the internal standard (1,2,4-trimethylbenzene) in toluene.

CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber vapor concentrations were determined with (all studies) and without (3-month and 2-year studies) animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. Based on experimental data, a T_{90} value of 12 minutes was selected for all studies.

The uniformity of cumene vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies; concentration uniformity with animals present in the chambers was measured once during the 2-week studies, once during the 3-month studies, and approximately quarterly in the 2-year studies. The vapor concentration was measured using an on-line gas chromatograph. Chamber concentration uniformity was maintained throughout the studies.

The persistence of cumene in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 4,000 ppm chambers in the 2-week studies and the 1,000 ppm chambers in the 3-month and 2-year studies with (all studies) and without (3-month and 2-year studies) animals present in the chambers. In the 2-week studies, the concentration decreased to 1% of the target concentration within 77 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 25 minutes without animals present and within 28 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 25 (rats) and 24 (mice) minutes without

animals present and within 35 (rats) and 27 (mice) minutes with animals present.

No evidence of degradation of cumene was noted in any part of the exposure system. With the exception of one peak noted in the distribution line samples taken during the 3-month studies, no impurity peaks were resolved with an area greater than 0.1% of the total peak area, and no additional impurities were detected with polar gas chromatography analyses. The relative purity of all generator reservoir and vapor trap samples exceeded 99% compared to the bulk chemical, and these samples were 99.97% pure by area percent.

2-WEEK STUDIES

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were approximately 4 to 5 weeks old. Animals were quarantined for 11 days and were approximately 5 to 6 weeks old on the first day of the studies. Before the studies began, four male and six female rats and five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At terminal sacrifice, serum was collected from five male and five female chamber control rats and mice, and serologic analyses were performed using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of five male and five female rats and mice were exposed to cumene at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 (rats) or 17 (mice) days. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded twice daily on exposure days for rats and mice. The animals were weighed initially, on days 6 and 13, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm , and stained with hematoxylin and eosin. Complete histopathologic examinations

were performed on all chamber control and 1,000 (mice only), 2,000, and 4,000 ppm animals, and tissues were examined to a no-effect level in the remaining exposure groups. Table 2 lists the tissues and organs routinely examined.

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to cumene and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were approximately 4 to 5 weeks old. Animals were quarantined for 11 (male rats and male and female mice) or 12 (female rats) days and were approximately 5 to 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Serologic analyses were performed by the study laboratory on five male and five female sentinel rats and mice 3 weeks after arrival at the study laboratory and five male and five female chamber control rats and mice at the end of the studies using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were exposed to cumene at concentrations of 0, 62.5, 125, 250, 500, or 1,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. One additional exposure day was scheduled during the last exposure week to give the rats at least two consecutive days of exposure before terminal sacrifice. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Animals were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 23 and from core study rats at the end of the study for hematology and clinical chemistry analyses; blood was collected from

the retroorbital sinus of mice at the end of the study for hematology analyses. Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Erythrocyte, leukocyte, and platelet counts; hemoglobin; packed red cell volume; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using an ABX Cobas Helios hematology analyzer (ABX Co., Irvine, CA). Manual hematocrit values were determined using a microcentrifuge (Heraeus Haemofuge, Germany) and a Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison to Helios values for packed cell volume. Blood smears for mice and rats were stained with Romanowsky-type aqueous stain in a Wescor 7100 Aerospray Slide Stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts for rats and mice were based on classifying a minimum of 100 white cells. Reticulocytes were stained with new methylene blue and enumerated as a reticulocyte:erythrocyte ratio using the Miller disc method (Brecher and Schneiderman, 1950). Blood samples for clinical chemistry analyses were placed in tubes containing separator gel and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Roche Cobas Fara (Roche Diagnostics, Branchburg, NJ). Table 2 lists the parameters measured.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on rats and mice exposed to 0, 125 (female mice only), 250, 500, or 1,000 ppm (rats and male mice). The parameters evaluated are listed in Table 2. Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, the left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. To quantify spermatogenesis, the testicular spermatid head count

was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus).

The left kidney was removed from all male and female core study rats at terminal necropsy, sectioned in half longitudinally, placed in a cassette, and fixed with 10% neutral buffered formalin for approximately 24 hours. After fixation, one half of the left kidney was processed and embedded in paraffin. A cross-section of small intestine was included in the embedding paraffin as a positive control for the cell proliferation study. After embedding, the left kidney was cut into three 5- μ m thick sections. The first section was stained with hematoxylin and eosin for histopathology (males and females). The second section was stained with Mallory-Heidenhain for evaluation for hyaline droplets (males and females). The third section was stained with proliferating cell nuclear antigen (PCNA) complexed with avidin and biotin for determination of cell proliferation indices (males). The right kidneys of all core study male rats were frozen and stored at approximately -70°C.

For male rats, the slides stained with PCNA were evaluated to determine the number of proximal tubule cells in the S-phase and the labeling index. Evaluation was done using a 20 \times objective and an ocular grid. Counting started at the second grid in from the outer edge of the cortex of the kidney. After one grid was counted, the slide was moved toward the medulla, and every other field encountered by the grid was counted. This procedure was repeated until at least 2,000 proximal tubule nuclei (labeled and unlabeled) were counted. If 2,000 proximal tubule nuclei were counted but the entire grid had not been counted, the remainder of the grid was counted. If 2,000 proximal tubule nuclei had not been counted by the time the outer medulla was reached, the slide was moved two grids laterally, and the counting process was resumed at the second grid in from the edge of the cortex.

The frozen kidneys from core study male rats were evaluated for α 2u-globulin and soluble protein. Each

right kidney was thawed; a volume of sodium/potassium phosphate buffer (pH ~7.2) equivalent to twice the recorded fresh weight of the sample was added, and the sample was homogenized for 30 to 60 seconds using an Ultra-Turrax tissue homogenizer (Tekmar Co., Cincinnati, OH). The homogenate was centrifuged at approximately 3,000 g for 15 minutes at 4° C. The protein content of each supernatant was measured in a 1:50 dilution (in phosphate-buffered saline-Tween) using the Bicinchoninic Acid Protein Assay Reagent kit (Pierce Chemical Co., Rockford, IL).

Analysis of α 2u-globulin in supernatants prepared from kidney homogenates was conducted using a competitive indirect enzyme-linked immunosorbent assay (ELISA). Ascites fluid containing anti- α 2u-globulin monoclonal antibodies was developed by Chemical Industry Institute of Toxicology (Research Triangle Park, NC). The amount of α 2u-globulin was measured by comparing the relative fluorescent signal intensity in the study samples to that observed with known amounts of α 2u-globulin present in calibration standards. Calibration standards and ELISA control standards (negative and positive) were plated in predetermined wells on 96-well microtiter plates. Calibration standards and study samples were assayed in triplicate.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (eyes were fixed in Davidson's solution for up to 10 days and then transferred to 10% neutral buffered formalin), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μ m, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all chamber control, 500 (female mice only), and 1,000 ppm animals, and tissues were examined to a no-effect level in the remaining exposure groups. Table 2 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to cumene at concentrations of 0, 125 (female mice only), 250, 500, or 1,000 (rats and male mice) ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Animals were quarantined for 11 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Cages, racks, and chambers were changed weekly. Cages were rotated weekly in chambers. Refer to Table 2 for more information about animal maintenance. Refer to Appendix J for information about feed composition and contaminants.

Clinical Examinations and Pathology

All animals were observed twice daily. For rats, clinical findings were recorded every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study. For mice, clinical findings were recorded weekly through week 13, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study. Rats and mice were weighed initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (eyes were fixed in Davidson's solution for up to 72 hours and then transferred to 10% neutral buffered formalin), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μ m, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System.

The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the eye, forestomach, lung, and nose of rats and mice, the kidney of male and female rats and male mice, the urinary bladder of male rats, and the liver of mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing

examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Cumene

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies 11 days	Rats: 11 days (males) or 12 days (females) Mice: 11 days	Rats: 11 days Mice: 10 days
Average Age When Studies Began 5 to 6 weeks	5 to 6 weeks	5 to 6 weeks
Date of First Exposure April 24, 2000	Rats: July 24 (males) or 25 (females), 2000 Mice: July 24, 2000	Rats: June 4, 2001 Mice: June 11, 2001
Duration of Exposure 6 hours plus T ₉₀ (12 minutes) per day, 5 days per week, for 16 (rats) or 17 (mice) days	6 hours plus T ₉₀ (12 minutes) per day, 5 days per week for 14 weeks	6 hours plus T ₉₀ (12 minutes) per day, 5 days per week for 105 weeks
Date of Last Exposure Rats: May 9, 2000 Mice: May 10, 2000	Rats: October 23 (males) or 24 (females), 2000 Mice: October 25 (males) or 26 (females), 2000	Rats: June 1-4, 2003 Mice: June 8-12, 2003
Necropsy Dates Rats: May 10, 2000 Mice: May 11, 2000	Rats: October 24 (males) or 25 (females), 2000 Mice: October 26 (males) or 27 (females), 2000	Rats: June 2-5, 2003 Mice: June 9-13, 2003
Average Age at Necropsy 8 to 9 weeks	19 to 20 weeks	Rats: 110 to 111 weeks Mice: 109 to 111 weeks
Size of Study Groups 5 males and 5 females	Rats: 10 males and 10 females (core study), 10 males and 10 females (clinical pathology study) Mice: 10 males and 10 females	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Cumene

2-Week Studies	3-Month Studies	2-Year Studies
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available <i>ad libitum</i> (except during animal exposure periods); changed weekly	Same as 2-week studies	Same as 2-week studies, except wafer form
Water		
Tap water (Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI); available <i>ad libitum</i>	Same as 2-week studies	Same as 2-week studies
Cages		
Stainless steel, wire bottom (Lab Products, Inc., Seaford, DE); changed weekly	Same as 2-week studies	Same as 2-week studies
Cageboard		
Untreated paper cage pan liner; changed daily	Same as 2-week studies	Untreated paper cage pan liner (Techboard, Sheperd Specialty Papers, Kalamazoo, MI); changed daily
Chamber Air Supply Filters		
Single HEPA (Environmental Filter, Santa Rosa, CA), changed annually; charcoal (RSE, Inc., New Baltimore, MI), new at study start; Purafil (Environmental Systems, Lynwood, WA), new at study start	Same as 2-week studies	Same as 2-week studies, except single HEPA was open stock
Chambers		
Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE); chambers changed weekly; excreta pans changed daily	Same as 2-week studies	Same as 2-week studies
Chamber Environment		
Temperature: 75° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Temperature: 75° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Temperature: 75° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour
Exposure Concentrations		
0, 250, 500, 1,000, 2,000, and 4,000 ppm	0, 62.5, 125, 250, 500, and 1,000 ppm	Rats: 0, 250, 500, and 1,000 ppm Mice: 0, 125 (females only), 250, 500, and 1,000 (males only) ppm

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Cumene

2-Week Studies	3-Month Studies	2-Year Studies
Type and Frequency of Observation Observed twice daily; animals were weighed initially, on days 6 and 13, and at the end of the studies; clinical findings were recorded twice daily on exposure days.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.	Observed twice daily; for rats, clinical findings were recorded every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study; for mice, clinical findings were recorded weekly through week 13, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study; animals were weighed initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies.
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 23 and from core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology. Hematology: hematocrit; packed red cell volume; hemoglobin; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials. Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Cumene

2-Week Studies	3-Month Studies	2-Year Studies
Histopathology Histopathology was performed on 0, 2,000, and 4,000 ppm rats and 0, 1,000, 2,000, and 4,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: kidney, liver, lung, and nose. These tissues were examined to a no-effect level in the remaining exposure groups.		
	Complete histopathology was performed on 0 and 1,000 ppm core study rats and 0, 500 (females only), and 1,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with mainstem bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the kidney of male rats in the remaining groups, the liver of 500 ppm male mice, and the forestomach of 250 ppm female mice were examined.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology None		
	At the end of the studies, sperm samples were collected from male animals in the 0, 250, 500, and 1,000 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females exposed to 0, 125 (mice), 250, 500, or 1,000 (rats) ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None
Renal Toxicity Study None		
	At the end of the study, concentrations of α 2u-globulin and soluble protein were measured in the right kidney of core study rats; the left kidneys were used for evaluation of hyaline droplets, assessment of cell proliferation indices, and histopathology.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., Harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk.

For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of $k=3$ was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as $1-P$ with the letter N added (e.g., $P=0.99$ is presented as $P=0.01N$).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, renal toxicity, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was

more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited

retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of cumene was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and increases in the frequency of micronucleated erythrocytes in rat bone marrow and mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally,

no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.*, 2000); negative results in this assay do not correlate

well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

2-WEEK STUDY

Rats exposed to 4,000 ppm cumene died during or after the first exposure on day 1, and half (2/5 males and 3/5 females) of the rats exposed to 2,000 ppm died by day 4 (Table 3). Final mean body weights and body weight gains of 2,000 ppm rats were significantly less than those of the chamber controls. Rats exposed to 2,000 ppm that died early were severely ataxic and lethargic on the day of exposure and into the following morning. Rats exposed to 1,000 ppm and those that survived exposure to 2,000 ppm exhibited varying degrees

of lethargy or ataxia, although the rats appeared to develop a tolerance, and the severity of clinical effects lessened by the end of week 1. Rats exposed to 500 ppm exhibited mild ataxia only after the initial exposure on day 1.

Significant increases in absolute and relative weights of the liver and relative weights of the kidney occurred in all exposed groups (Table G1). The absolute kidney weights of 250 and 1,000 ppm males and 250, 500,

TABLE 3
Survival and Body Weights of Rats in the 2-Week Inhalation Study of Cumene

Concentration (ppm)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	82 ± 2	146 ± 4	64 ± 4	
250	5/5	84 ± 2	149 ± 4	65 ± 3	103
500	5/5	83 ± 3	148 ± 7	66 ± 5	102
1,000	5/5	84 ± 2	148 ± 5	63 ± 3	101
2,000	3/5 ^c	85 ± 3	102 ± 3**	15 ± 3**	70
4,000	0/5 ^d	86 ± 2	—	—	—
Female					
0	5/5	79 ± 2	120 ± 3	41 ± 1	
250	5/5	78 ± 2	122 ± 3	43 ± 2	101
500	5/5	78 ± 2	125 ± 1	47 ± 2	104
1,000	5/5	80 ± 2	122 ± 3	43 ± 2	102
2,000	2/5 ^e	79 ± 3	93 ± 3**	10 ± 2**	78
4,000	0/5 ^d	79 ± 3	—	—	—

** Significantly different ($P \leq 0.01$) from the chamber control group by Dunnett's test

^a Number of animals surviving at 2 weeks/number initially in group

^b Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

^c Days of death: 2, 4

^d Day of deaths: 1

^e Days of death: 2, 3, 4

and 1,000 ppm females were significantly increased. Absolute and relative thymus weights were significantly decreased in the 2,000 ppm groups. No exposure-related gross lesions were observed.

Accumulation of minimal to mild hyaline droplets was observed in the renal tubular cortex of males exposed to 250 to 2,000 ppm, and the incidences were greatest in the 250 and 1,000 ppm groups [chamber controls, 0/5; 250 ppm, 3/5 (1.3); 500 ppm, 2/5 (1.5); 1,000 ppm, 3/5 (1.0); 2,000 ppm, 1/5 (1.0); 4,000 ppm, 0/5]. The lack of hyaline droplets in 2,000 ppm males that died on days 2

and 4 and 4,000 ppm males that died on day 1 was considered the result of the short period of exposure.

Exposure Concentration Selection Rationale: In male rats, increased incidences of hyaline droplet accumulation in the renal cortex were caused by cumene exposure. There was no evidence of other renal tubule epithelium damage. Based on the mortalities at 2,000 and 4,000 ppm, organ weight changes, and the lack of lesions in tissues examined, cumene exposure concentrations selected for the 3-month inhalation study in rats were 62.5, 125, 250, 500, and 1,000 ppm.

3-MONTH STUDY

All rats survived to the end of the study (Table 4). Final mean body weights and body weight gains of all exposed groups were similar to those of the chamber controls. Male rats exposed to 1,000 ppm exhibited ataxia for several days but recovered and appeared to develop a tolerance by day 3.

The hematology and clinical chemistry data for rats in the 3-month inhalation study of cumene are listed in Tables 5 and F1. The most consistent changes involved markers of hepatic injury/function. Serum total bile acid concentrations were significantly increased in essentially all exposed groups on days 3 and 23 of the study. The bile acid increases occurred in an exposure concentration-related manner (ranging from approximately 20% in the 62.5 ppm groups to approximately 65% in the 1,000 ppm groups). This effect was, however, transient and by study termination affected only the 500 and 1,000 ppm males. Markers of hepatocyte injury, alanine aminotransferase and sorbitol dehydrogenase activities,

demonstrated an exposure concentration-related decrease in serum activity. Alanine aminotransferase appeared to be most affected, demonstrating some effect at all time points; the sorbitol dehydrogenase effect was limited to week 14. The magnitude of the effect intensified with time, and by study termination, the 250, 500, and 1,000 ppm male and female groups demonstrated decreases in serum activity of these two enzymes (up to an approximate 58% decrease in alanine aminotransferase activity in the 1,000 ppm males and females). Serum alkaline phosphatase activity (a marker of hepatobiliary function) also demonstrated minimal exposure concentration-related decreases; the decreases were significant in male rats exposed to 250 ppm or greater at week 14 and in 500 and 1,000 ppm females at all time points. No exposure-related lesions were observed in the livers of exposed rats. However, the relative liver weights increased with increasing exposure concentrations in essentially all male and female groups (Tables 6 and G2). The liver weight effects, coupled with the effects detected in the clinical chemistry, could suggest hepatic effects that altered liver function, resulting in

TABLE 4
Survival and Body Weights of Rats in the 3-Month Inhalation Study of Cumene

Concentration (ppm)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	89 ± 3	302 ± 8	213 ± 9	
62.5	10/10	91 ± 3	303 ± 6	212 ± 6	100
125	10/10	90 ± 3	313 ± 5	223 ± 6	104
250	10/10	91 ± 3	319 ± 4	228 ± 5	105
500	10/10	87 ± 3	303 ± 5	216 ± 6	100
1,000	10/10	89 ± 2	312 ± 5	223 ± 5	103
Female					
0	10/10	84 ± 2	190 ± 2	105 ± 3	
62.5	10/10	84 ± 2	184 ± 3	100 ± 3	97
125	10/10	81 ± 1	190 ± 4	109 ± 4	100
250	10/10	83 ± 2	187 ± 3	104 ± 3	99
500	10/10	85 ± 2	182 ± 3	97 ± 3	96
1,000	10/10	83 ± 2	184 ± 3	101 ± 3	97

^a Number of animals surviving at 3 months/number initially in group

^b Weights and weight changes are given as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test.

TABLE 5
Selected Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10	10	10
Male						
Albumin (g/dL)						
Day 3	4.0 ± 0.1	3.8 ± 0.1	3.6 ± 0.1*	3.6 ± 0.1**	3.8 ± 0.1	3.8 ± 0.1
Day 23	3.8 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.0
Week 14	3.8 ± 0.1	3.9 ± 0.0	3.8 ± 0.0	3.9 ± 0.0	4.1 ± 0.1**	4.2 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 3	64 ± 2	61 ± 1	61 ± 1	64 ± 2	60 ± 2	56 ± 1**
Day 23	44 ± 1	39 ± 1*	39 ± 1**	38 ± 1**	35 ± 1**	35 ± 0**
Week 14	113 ± 6	113 ± 11	110 ± 12	70 ± 4**	61 ± 3**	50 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	733 ± 14	759 ± 16	779 ± 14	794 ± 24	771 ± 24	674 ± 15
Day 23	496 ± 15	490 ± 11	506 ± 15	485 ± 15	456 ± 27	470 ± 12
Week 14	309 ± 7	294 ± 4	293 ± 10	283 ± 9*	275 ± 7**	250 ± 6**
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 1	12 ± 1	12 ± 1	11 ± 1	12 ± 0	11 ± 1
Day 23	13 ± 0	13 ± 0	13 ± 0	12 ± 1	12 ± 0	12 ± 0
Week 14	26 ± 1	24 ± 2	22 ± 1	20 ± 1**	17 ± 1**	17 ± 1**
Bile acids (μmol/L)						
Day 3	33.8 ± 1.1	39.9 ± 0.9**	47.1 ± 0.7**	50.9 ± 1.7**	50.4 ± 2.0**	55.6 ± 4.1**
Day 23	32.1 ± 2.9	33.2 ± 1.4	37.7 ± 1.4*	41.1 ± 1.0**	43.4 ± 1.7**	44.2 ± 1.4**
Week 14	28.6 ± 1.7	31.2 ± 1.3	32.7 ± 1.5	30.9 ± 0.9	32.4 ± 0.9*	35.9 ± 2.2**
Female						
Albumin (g/dL)						
Day 3	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.6 ± 0.0	3.7 ± 0.1
Day 23	3.8 ± 0.0	3.8 ± 0.0	3.7 ± 0.0	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
Week 14	4.2 ± 0.1	4.0 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.6 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 3	50 ± 1	51 ± 2	48 ± 2	48 ± 2	47 ± 2	42 ± 2**
Day 23	37 ± 1	37 ± 1	34 ± 1	35 ± 1	34 ± 1	33 ± 1
Week 14	80 ± 8	64 ± 6	63 ± 4	53 ± 4**	53 ± 3**	33 ± 1**
Alkaline phosphatase (IU/L)						
Day 3	642 ± 18	636 ± 23	618 ± 19	608 ± 12	585 ± 15*	474 ± 10**
Day 23	372 ± 6	388 ± 11	370 ± 8	362 ± 9	348 ± 7*	319 ± 7**
Week 14	272 ± 9	275 ± 8	262 ± 12	267 ± 7	243 ± 7*	207 ± 6**
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 1	13 ± 0	13 ± 0	12 ± 0	12 ± 0	12 ± 0
Day 23	12 ± 1	11 ± 0	11 ± 0	12 ± 0	11 ± 0	12 ± 0
Week 14	22 ± 1	21 ± 1	19 ± 0	19 ± 1	18 ± 1**	15 ± 1**
Bile acids (μmol/L)						
Day 3	27.2 ± 1.3	32.6 ± 1.9*	34.4 ± 1.8**	38.5 ± 1.8**	42.5 ± 0.9**	38.0 ± 1.7**
Day 23	23.0 ± 0.7	27.6 ± 1.5**	29.1 ± 0.9**	31.5 ± 1.1**	38.5 ± 2.9**	38.4 ± 3.6**
Week 14	29.7 ± 2.6	24.3 ± 1.3	26.2 ± 1.6	32.1 ± 4.3	25.7 ± 2.3	23.8 ± 0.9

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test

** $P \leq 0.01$

^a Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

TABLE 6
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats
in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	312 ± 8	313 ± 6	322 ± 5	331 ± 4	314 ± 5	323 ± 5
R. Kidney						
Absolute	0.923 ± 0.024	0.980 ± 0.025	1.010 ± 0.031 ^{*b}	1.059 ± 0.021 ^{**}	1.070 ± 0.017 ^{**}	1.152 ± 0.023 ^{**}
Relative	2.962 ± 0.032	3.128 ± 0.051 ^{**}	3.131 ± 0.056 ^{**b}	3.194 ± 0.036 ^{**}	3.411 ± 0.045 ^{**}	3.561 ± 0.029 ^{**}
Liver						
Absolute	9.518 ± 0.327	10.123 ± 0.267	10.260 ± 0.264	11.170 ± 0.302 ^{**}	11.589 ± 0.282 ^{**}	12.637 ± 0.288 ^{**}
Relative	30.481 ± 0.348	32.279 ± 0.278 [*]	31.792 ± 0.430 [*]	33.660 ± 0.558 ^{**}	36.895 ± 0.563 ^{**}	39.068 ± 0.549 ^{**}
Female						
Necropsy body wt	195 ± 2	190 ± 3	194 ± 4	190 ± 3	185 ± 3	187 ± 4
R. Kidney						
Absolute	0.637 ± 0.016	0.636 ± 0.010	0.649 ± 0.018	0.655 ± 0.017	0.645 ± 0.011	0.675 ± 0.011
Relative	3.263 ± 0.061	3.355 ± 0.049	3.322 ± 0.057	3.439 ± 0.057 [*]	3.486 ± 0.044 ^{**}	3.612 ± 0.040 ^{**}
Liver						
Absolute	5.553 ± 0.130	5.669 ± 0.148	5.885 ± 0.204	5.959 ± 0.137	5.979 ± 0.133	6.923 ± 0.227 ^{**}
Relative	28.442 ± 0.389	29.858 ± 0.458	30.094 ± 0.634 [*]	31.289 ± 0.412 ^{**}	32.286 ± 0.386 ^{**}	36.958 ± 0.724 ^{**}

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

^b n=9

some perturbation in bile acid uptake or excretion and liver enzyme production/turnover or, possibly, inhibition. Serum albumin concentration (a nonspecific marker of hepatic function) was either unaffected or slightly (approximately 10%) increased in exposed males and females (Tables 5 and F1). The liver synthesizes albumin, and these results suggest that albumin synthesis was unaffected by exposure to cumene.

No exposure-related gross lesions were observed. Relative kidney and liver weights of all exposed groups of males, relative liver weights of 125 ppm or greater females, and relative kidney weights of 250 ppm or greater females were greater than those of the chamber controls (Tables 6 and G2). In addition, the absolute weights of the kidney were significantly increased in 125 ppm or greater males, and the absolute weights of the liver were increased in 250 ppm or greater males and 1,000 ppm females. There were no significant differences between exposed and chamber control males in reproductive tissue evaluations (Table H1). Exposed females differed significantly from the chamber control females in the relative length of time spent in estrus and proestrus (Table H2).

In cell proliferation analyses of the left kidney, the mean numbers of proximal tubule cells in the S-phase were significantly increased in the 500 and 1,000 ppm groups; however, the number of cells labeled and the labeling index were not significantly different from the chamber control group (Table 7). Concentrations of soluble protein and α 2u-globulin in the right kidney from male rats demonstrated increases in all exposed groups. Soluble protein levels were significantly increased in male rats exposed to 250 ppm or greater. Amounts and concentrations of α 2u-globulin were significantly increased in groups exposed to 125 ppm or greater. These findings are consistent with the hyaline droplet accumulations observed microscopically (Table 8).

The severities of hyaline droplet accumulation in the renal cortical tubules of exposed male rats were

increased, and the incidences and severities of renal cortical tubule regeneration were generally slightly increased (Table 8). These changes were accompanied by significantly increased incidences of medullary granular casts in groups exposed to 250 ppm or greater. Hyaline droplet accumulation was observed in all male rats and was minimal to mild in the chamber control and 62.5 ppm groups, minimal to moderate at 125 ppm, and mild to moderate at 250 ppm or greater. In males, minimal tubular regeneration occurred in the chamber controls; minimal to mild lesions were observed in groups exposed to 62.5 or 125 ppm, and minimal to moderate tubular regeneration occurred in groups exposed to 250 ppm or greater. In males, minimal casts were observed in the 125 ppm group; minimal to moderate casts occurred in the 250 ppm group, and minimal to marked casts occurred in groups exposed to 500 or 1,000 ppm. In hematoxylin and eosin stained sections, hyaline droplets appeared as brightly eosinophilic globules of varying size in the renal cortical tubular epithelium. In Mallory-Heidenhain stained sections, hyaline droplets were magenta and were more easily visualized for severity evaluations. Tubular regeneration was characterized by tubules lined by more basophilic epithelium and having larger nuclei than the surrounding tubules. Affected tubules typically occurred in clusters. The granular casts were generally present at the cortico-medullary junction and caused dilation of the tubular lumen with lightly eosinophilic granular acellular material.

Exposure Concentration Selection Rationale: In male rats, granular casts in the renal tubules of the medulla and increased severities of renal tubule regeneration and hyaline droplet accumulation in the cortex were caused by cumene exposure. The granular casts and renal tubule regeneration are indicative of some renal tubule epithelium damage. Based on the lack of mortalities and body weight effects, minimal organ weight changes, and the lack of lesions in other tissues, cumene exposure concentrations selected for the 2-year inhalation study in rats were 250, 500, and 1,000 ppm.

TABLE 7
Renal Toxicity Data for Male Rats in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10	10	10
Cells labeled	60.60 ± 4.25	78.90 ± 5.60	63.80 ± 6.03	54.90 ± 4.50	43.70 ± 6.21	79.40 ± 6.65
Cells counted	2,095 ± 25	2,190 ± 28	2,100 ± 29	2,171 ± 31	2,268 ± 42	2,223 ± 45
Labeling index (%) ^b	2.894 ± 0.204	3.607 ± 0.266	3.051 ± 0.305	2.536 ± 0.219	1.944 ± 0.306	3.614 ± 0.346
Soluble protein (mg/mL)	21.22 ± 1.27	23.43 ± 0.51	23.93 ± 0.93 ^c	25.36 ± 0.69*	25.51 ± 0.73**	26.16 ± 1.09**
α2u-Globulin (nmol/g kidney)	172.2 ± 22.3	328.1 ± 69.6	383.4 ± 46.3** ^c	420.7 ± 50.1**	363.2 ± 41.4**	575.2 ± 74.8**
α2u-Globulin (ng/μg soluble protein)	76.46 ± 9.24	130.98 ± 27.27	150.90 ± 19.23* ^c	154.35 ± 16.98**	133.01 ± 14.25**	209.79 ± 31.34**

* Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test

** $P \leq 0.01$

^a Data are presented as mean ± standard error.

^b Labeling index was calculated as the number of labeled cells divided by the total number of cells counted times 100. A minimum of 2,000 cells were counted.

^c n=9

TABLE 8
Incidences of Nonneoplastic Lesions in the Kidney of Male Rats in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Number Examined Microscopically	10	10	10	10	10	10
Cortex Renal Tubule, Accumulation, Hyaline Droplet ^a	10 (1.1) ^b	10 (1.4)	10 (1.9)	10 (2.4)	10 (3.0)	10 (2.9)
Cortex Renal Tubule, Regeneration	8 (1.0)	6 (1.2)	8 (1.5)	10 (1.8)	10 (2.1)	10 (2.1)
Medulla, Casts Granular	0	0	2 (1.0)	8** (1.5)	10** (2.5)	9** (2.2)

** Significantly different ($P \leq 0.01$) from the chamber control group by the Fisher exact test

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 9 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of rats was similar to that of the chamber controls.

Body Weights and Clinical Findings

Mean body weights of exposed groups of males were similar to those of the chamber controls throughout the study; mean body weights of 1,000 ppm females were slightly less than those of the chamber controls during the second year of the study but were similar to the

chamber controls at the end of the study (Tables 10 and 11; Figure 3). There were no clinical findings related to exposure to cumene. Seizures occurred in a few exposed males (chamber control, 0/50; 250 ppm, 1/50; 500 ppm, 4/50; 1,000 ppm, 1/50) and in a few chamber control and exposed females (6/50, 8/50, 5/50, 5/50). The seizures were clonic and of short duration. They were most frequently observed and recorded during daily animal care activities. The first seizure episode was observed in a female rat at 32 weeks of exposure. No evidence of brain lesions was found to account for the cause or effect of the clonic seizures noted clinically in the animals. Similar, sporadic seizures have been observed in F344/N rats in six other NTP inhalation or dermal studies at

TABLE 9
Survival of Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	20	24	21	24
Natural deaths	4	3	2	2
Animals surviving to study termination	26	23	27	24
Percent probability of survival at end of study ^a	52	46	54	48
Mean survival (days) ^b	676	665	671	684
Survival analysis ^c	P=0.994N	P=0.511	P=1.000N	P=0.946
Female				
Animals initially in study	50	50	50	50
Moribund	23	18	17	15
Natural deaths	6	5	2	3
Animals surviving to study termination	21	27	31	32
Percent probability of survival at end of study	42	54	62	64
Mean survival (days)	673	677	685	684
Survival analysis	P=0.061N	P=0.357N	P=0.109N	P=0.071N

^a Kaplan-Meier determinations

^b Mean of all deaths (uncensored, censored, and terminal sacrifice)

^c The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by N.

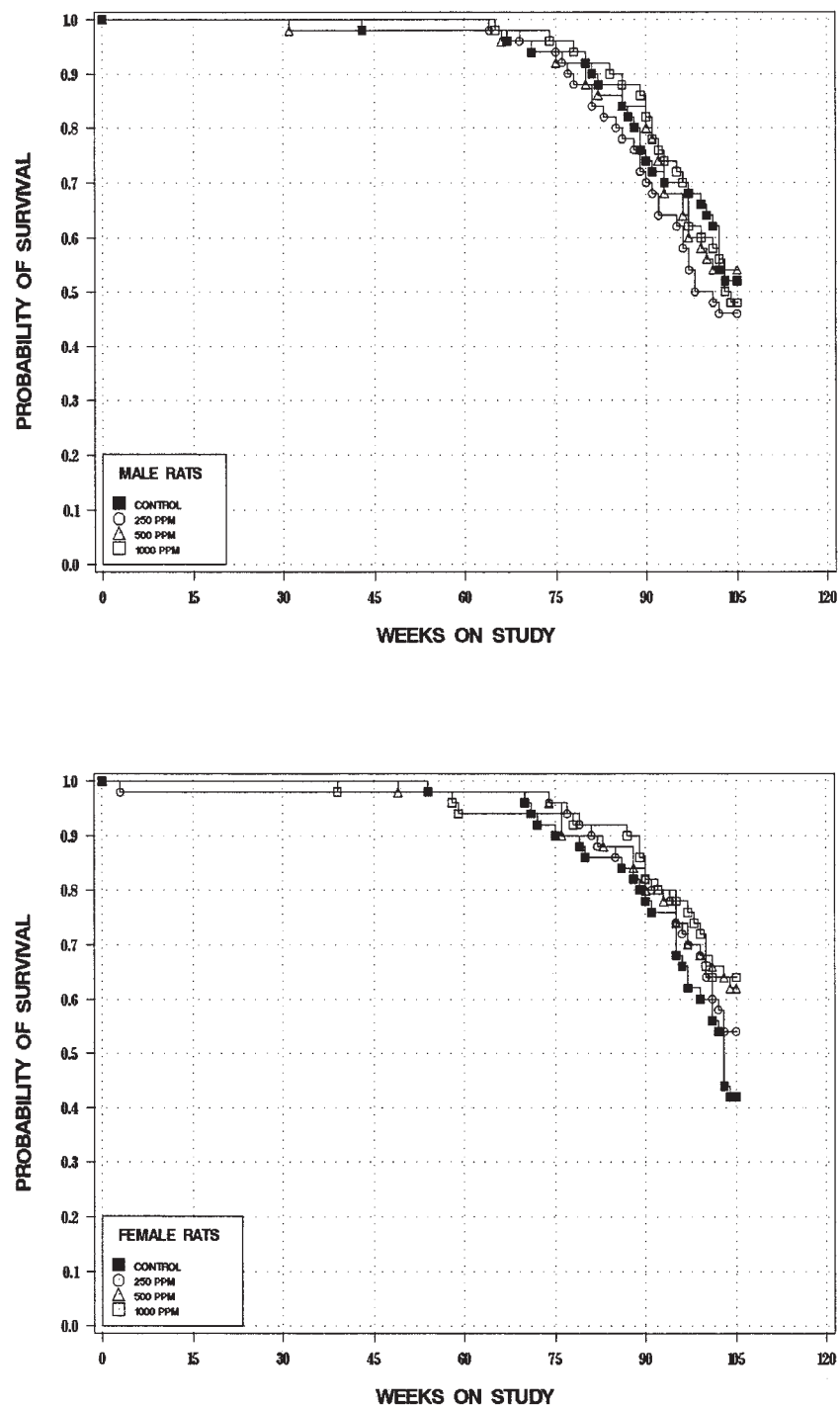


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats
Exposed to Cumene by Inhalation for 2 Years

TABLE 10
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Cumene

Days on Study	Chamber Control		250 ppm			500 ppm			1,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	107	50	106	99	50	106	99	50	105	97	50
9	145	50	144	99	50	142	98	50	137	94	50
16	176	50	175	99	50	174	99	50	169	96	50
23	202	50	204	101	50	203	101	50	198	98	50
30	224	50	226	101	50	227	101	50	221	98	50
37	245	50	247	101	50	248	101	50	243	99	50
44	260	50	261	100	50	264	101	50	258	99	50
51	275	50	275	100	50	278	101	50	272	99	50
58	288	50	289	100	50	292	101	50	287	100	50
65	300	50	302	100	50	305	101	50	301	100	50
72	311	50	311	100	50	316	102	50	310	100	50
79	321	50	321	100	50	326	102	50	321	100	50
86	328	50	328	100	50	336	103	50	330	101	50
114	363	50	360	99	50	368	101	50	362	100	50
142	391	50	385	99	50	394	101	50	388	99	50
170	415	50	411	99	50	419	101	50	408	98	50
198	432	50	427	99	50	434	101	50	423	98	50
226	446	50	442	99	50	449	101	49	441	99	50
254	461	50	455	99	50	464	101	49	456	99	50
282	473	50	467	99	50	476	101	49	465	98	50
310	480	49	474	99	50	485	101	49	473	99	50
338	490	49	482	98	50	490	100	49	484	99	50
366	496	49	490	99	50	499	100	49	492	99	50
394	507	49	500	99	50	508	100	49	500	99	50
422	513	49 ^a	509	99	50	517	101	49	504	98	50
450	519	49	514	99	49	518	100	49	504	97	50
478	523	48	510	98	49	520	100	48	511	98	49
506	531	47	523	99	48	527	99	47	517	97	49
534	534	47	521	98	46	529	99	46	515	97	48
562	536	46	528	99	44	534	100	44	523	98	46
591	540	44	534	99	40	532	99	43	524	97	45
618	540	40	529	98	38	537	99	42	517	96	44
646	539	36	534	99	32	534	99	36	524	97	38
660	541	35	524	97	32	535	99	34	517	96	37
674	532	35	525	99	29	534	100	32	514	96	35
688	529	34	541	102	25	529	100	30	515	97	31
702	527	32	533	101	25	527	100	28	509	97	30
716	531	27	536	101	23	527	99	27	500	94	28
Mean for weeks											
1-13	245		245	100		247	101		242	99	
14-52	439		434	99		442	101		433	99	
53-103	527		522	99		525	100		512	97	

^a Number of animals weighed was less than number of animals surviving.

TABLE 11
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Cumene

Days on Study	Chamber Control		250 ppm			500 ppm			1,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	89	50	89	100	50	88	99	50	88	99	50
9	110	50	110	100	50	110	100	50	107	98	50
16	124	50	124	100	49	125	101	50	123	99	50
23	135	50	138	102	49	138	102	50	137	101	50
30	147	50	150	102	49	149	101	50	149	101	50
37	157	50	160	102	49	159	101	50	158	101	50
44	164	50	167	102	49	166	101	50	166	101	50
51	171	50	172	101	49	171	100	50	171	100	50
58	174	50	176	101	49	175	100	50	175	100	50
65	180	50	182	101	49	180	100	50	180	100	50
72	183	50	186	102	49	185	101	50	183	100	50
79	188	50	191	102	49	189	101	50	188	100	50
86	191	50	193	101	49	193	101	50	190	100	50
114	204	50	208	102	49	205	100	50	202	99	50
142	215	50	217	101	49	216	100	50	211	98	50
170	227	50	231	102	49	227	100	50	222	98	50
198	234	50	236	101	49	233	100	50	227	97	50
226	241	50	243	101	49	240	100	50	233	97	50
254	251	50	253	101	49	248	99	50	239	95	50
282	259	50	260	100	49 ^a	255	98	50	246	95	49
310	269	50	270	100	49	262	98	50	251	94	49
338	281	50	282	100	49	269	96	50	259	92	49
366	294	50	293	100	49	282	96	49	269	91	49
394	302	49	304	101	49	292	97	49	279	92	49
422	312	49	316	101	49	306	98	49	291	93	47
450	318	49	320	101	49	312	98	49	298	94	47
478	323	49	326	101	49	317	98	49	303	94	47
506	336	46	338	101	49	326	97	49	314	94	47
534	341	45	344	101	48	330	97	45	320	94	47
562	346	43	345	100	46	336	97	45	327	95	46
591	352	43	354	100	44	341	97	44	334	95	46
618	355	41	361	102	42	343	97	42	333	94	45
646	354	38	362	102	40	346	98	39	339	96	40
660	349	38	364	104	39	348	100	39	341	98	39
674	350	33	366	104	35	350	100	36	342	98	39
688	348	30	363	104	35	349	100	35	344	99	37
702	343	30	370	108	32	351	102	34	348	101	33
716	344	26	373	108	28	356	104	32	351	102	32
Mean for weeks											
1-13	155		157	101		156	101		155	100	
14-52	242		244	101		239	99		232	96	
53-103	335		344	103		330	99		321	96	

^a Number of animals weighed was less than number of animals surviving.

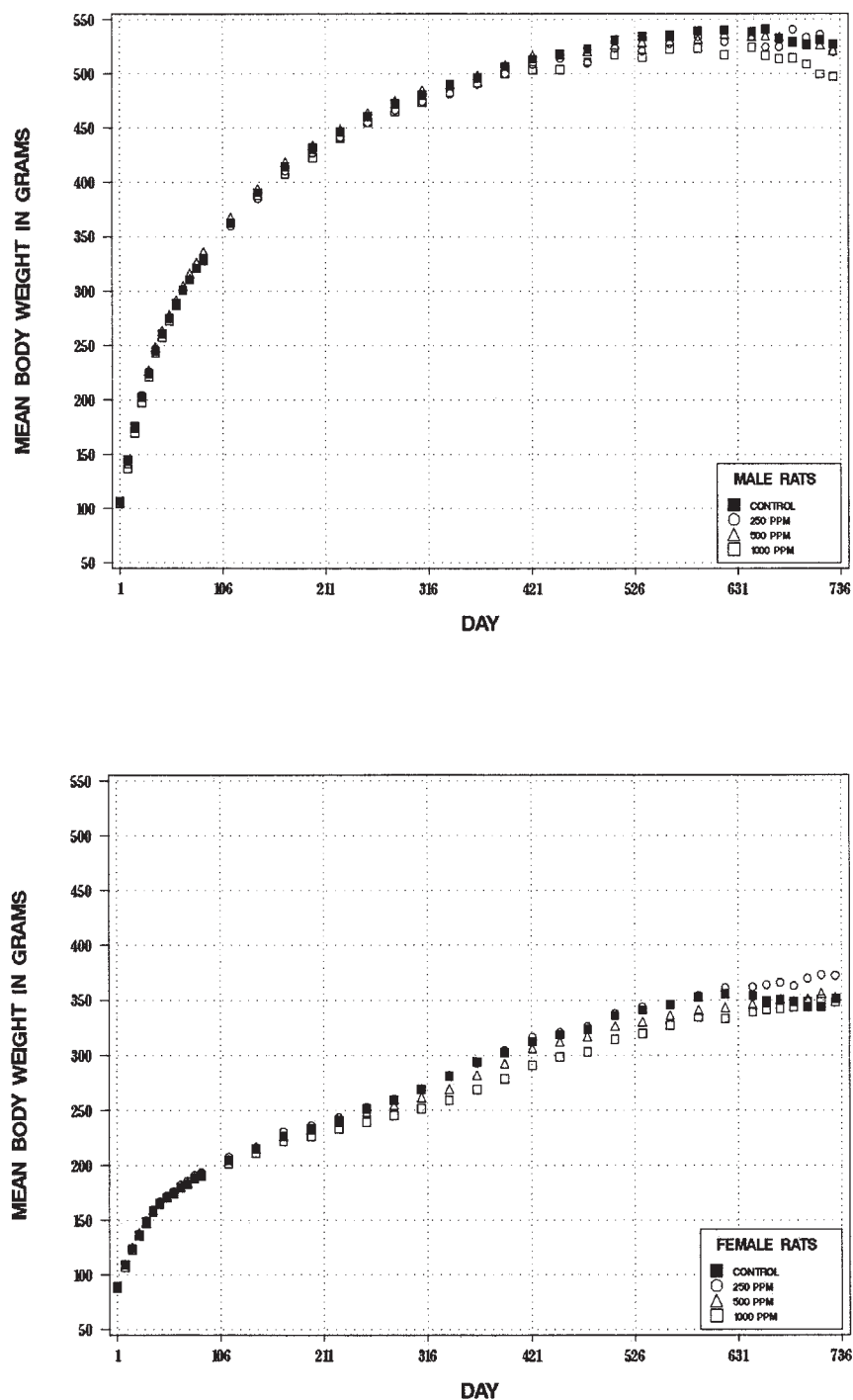


FIGURE 3
Growth Curves for Male and Female Rats
Exposed to Cumene by Inhalation for 2 Years

three different laboratories. In all of these studies, the single common factor was that the animals were housed individually. No such episodes have been observed in concurrent dosed feed, gavage, or drinking water studies in which animals are group housed. In the individually housed animals, most seizures were observed early in the day when technical and maintenance activities were

commencing following the animals' dark cycle period. No deaths were associated with the seizures, and there were no correlations with body weight, feed consumption or composition, or histopathologic lesions in this or the other studies. Thus, these transient events were not considered to have affected the toxicologic or carcinogenic evaluations of this study.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the nose, kidney, and testis. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Nose: Exposure to cumene resulted in increased incidences of adenoma of the respiratory epithelium, respiratory epithelium hyperplasia, olfactory epithelium basal cell hyperplasia, and goblet cell hyperplasia (Tables 12, A1, A2, A4, B1, B2, and B4). These nasal lesions were seen in all three nasal cavity levels that are routinely examined in NTP toxicity and carcinogenicity studies. Level I is excised immediately posterior to the upper incisor teeth; Level II is excised through the level of the incisive papilla anterior to the first palatal ridge, and Level III is excised through the middle of the second molar teeth. Levels I and II contain the naso- and maxilloturbinates that, along with the nasal passages (meatuses) and septum, are primarily lined by ciliated respiratory type epithelium. Level III encompasses the olfactory region of the nose, with ethmoid turbinates and meatuses lined entirely by specialized olfactory neuroepithelium (Boorman *et al.*, 1990).

Incidences of adenoma of the respiratory epithelium occurred with a positive trend in males and were significantly increased in all exposed groups of males and in 250 ppm females; the incidences in all exposed groups of rats exceeded the ranges for historical chamber controls in inhalation studies and historical controls (all routes) (Tables 12, A2, A3a, B2, and B3). The incidences of multiple adenomas in the respiratory epithelium of exposed groups of males increased with increasing exposure concentration, and the incidence in the 1,000 ppm group was significantly increased. Microscopically, the adenomas were rounded masses or papillary projections arising from the turbinates or the lateral wall and protruding into the nasal cavity at section Levels I and II (Plate 1). The adenomas consisted of cords or clusters of basophilic cuboidal epithelial cells that often formed acinar patterns (Plates 2 and 3).

Incidences of hyperplasia of basal cells in the olfactory epithelium were significantly increased in all exposed

groups (Tables 12, A4, and B4). Basal cell hyperplasia was characterized by increased numbers of basal cells crowded along the basement membrane of the olfactory epithelium, most often affecting cells lining the nasal septum (Plates 4 and 5). In more severe lesions, affected basal cells infiltrated the overlying epithelium and occasionally formed small rosettes within the olfactory epithelium.

The incidences of hyperplasia of the respiratory epithelium were significantly increased in all exposed groups of males, and the incidence of this lesion was significantly increased in 1,000 ppm females (Tables 12, A4, and B4). Hyperplasia of the respiratory epithelium and adenoma form a morphologic continuum. Hyperplastic lesions consisted of minimal to marked increases in populations of homogeneous respiratory epithelial cells (Plate 6) with less distinct acinar formation than adenomas.

The incidence of goblet cell hyperplasia was significantly increased in 250 ppm males (Tables 12 and A4). Goblet cell hyperplasia was characterized by minimal to moderate increases in the number of mucous cells in respiratory epithelium. Hyperplasia was often accompanied by enlargement or hypertrophy of the mucous cells. The hyperplasia resulted in thickening of the respiratory epithelium and an undulating surface. The affected cells were taller, with many large cells containing abundant amounts of mucin. The nuclei appeared more numerous. Similar goblet cell hyperplasia with hypertrophy was observed in the olfactory epithelium of a 500 ppm male rat (Plate 7).

Kidney: The incidences of renal tubule adenoma were increased in all exposed groups of males compared to the chamber control group and exceeded the historical range for chamber controls in inhalation studies (Tables 13, A2, and A3b). The incidences of renal tubule carcinoma were increased in 500 and 1,000 ppm males and exceeded the historical chamber control range; one 500 ppm male rat had a bilateral renal tubule carcinoma. The incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 500 ppm males, and the incidences of these combined lesions in all exposed groups of males exceeded the range for historical chamber controls.

Six of the renal tubule adenomas were large enough to be observed grossly at necropsy and were described as nodules or lesions varying in size from 1 to 2 mm in

TABLE 12
Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	49	50
Olfactory Epithelium, Hyperplasia, Basal Cell ^a	0	19** (1.1) ^b	27** (1.0)	26** (1.0)
Respiratory Epithelium, Hyperplasia	0	15** (2.0)	16** (2.9)	23** (2.7)
Goblet Cell, Hyperplasia	3 (1.7)	11* (2.3)	7 (2.3)	5 (2.0)
Glands, Respiratory Epithelium, Adenoma	0	0	1	0
Respiratory Epithelium, Adenoma, Multiple	0	1	2	6*
Respiratory Epithelium, Adenoma (includes multiple and all sites) ^c				
Overall rate ^d	0/50 (0%)	7/50 (14%)	18/49 (37%)	10/50 (20%)
Adjusted rate ^e	0.0%	17.6%	43.2%	23.3%
Terminal rate ^f	0/26 (0%)	5/23 (22%)	13/27 (48%)	7/24 (29%)
First incidence (days)	— ^g	639	638	674
Poly-3 test ^h	P=0.004	P=0.006	P<0.001	P<0.001
Female				
Number Examined Microscopically	50	48	50	50
Olfactory Epithelium, Hyperplasia, Basal Cell	0	14** (1.0)	25** (1.0)	31** (1.1)
Respiratory Epithelium, Hyperplasia	0	0	4 (3.0)	6* (2.3)
Respiratory Epithelium, Adenoma ⁱ				
Overall rate	0/50 (0%)	5/48 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	0.0%	12.2%	9.3%	6.9%
Terminal rate	0/21 (0%)	5/27 (19%)	4/31 (13%)	2/32 (6%)
First incidence (days)	—	730 (T)	730 (T)	638
Poly-3 test	P=0.320	P=0.030	P=0.066	P=0.130

(T) Terminal sacrifice

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

^c Historical incidence for 2-year inhalation studies with chamber control groups (mean \pm standard deviation): 1/447 (0.2% \pm 0.7%), range 0%-2%; all routes: 2/1,439 (0.1% \pm 0.5%), range 0%-2%

^d Number of animals with neoplasm per number of animals with nose examined microscopically

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g Not applicable; no neoplasms in animal group

^h Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

ⁱ Historical incidence for inhalation studies: 0/496; all routes: 0/1,343

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia ^a	0	3 (3.3) ^b	8** (2.6)	6* (2.2)
Papilla, Mineralization	5 (1.0)	35** (1.7)	44** (2.1)	41** (2.1)
Pelvis, Transitional Epithelium, Hyperplasia	3 (1.7)	5 (1.8)	14** (2.4)	15** (2.0)
Nephropathy	47 (2.3)	47 (2.6)	47 (2.9)	50 (2.7)
Renal Tubule, Adenoma ^c				
Overall rate ^d	1/50 (2%)	4/50 (8%)	5/50 (10%)	4/50 (8%)
Adjusted rate ^e	2.4%	10.0%	12.1%	9.3%
Terminal rate ^f	1/26 (4%)	1/23 (4%)	2/27 (7%)	2/24 (8%)
First incidence (days)	729 (T)	665	679	635
Poly-3 test ^g	P=0.219	P=0.165	P=0.099	P=0.187
Renal Tubule, Carcinoma, Bilateral	0	0	1	0
Renal Tubule, Carcinoma (includes bilateral) ^h				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.4%	2.5%	7.3%	7.0%
Terminal rate	1/26 (4%)	1/23 (4%)	2/27 (7%)	2/24 (8%)
First incidence (days)	729 (T)	729 (T)	639	618
Poly-3 test	P=0.180	P=0.749	P=0.302	P=0.314
Renal Tubule, Adenoma or Carcinoma ⁱ				
Overall rate	2/50 (4%)	5/50 (10%)	8/50 (16%)	7/50 (14%)
Adjusted rate	4.8%	12.5%	19.2%	16.2%
Terminal rate	2/26 (8%)	2/23 (9%)	4/27 (15%)	4/24 (17%)
First incidence (days)	729 (T)	665	639	618
Poly-3 test	P=0.087	P=0.198	P=0.044	P=0.087
Renal Tubule, Lipoma ^j	1	0	0	1
Female				
Number Examined Microscopically	50	50	50	50
Nephropathy	38 (1.4)	37 (1.5)	41 (1.9)	44 (1.9)

(T) Terminal sacrifice

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

^c Historical incidence for 2-year inhalation studies with chamber control groups (mean \pm standard deviation): 4/449 (0.9% \pm 1.0%), range 0%-2%; all routes: 8/1,436 (0.6% \pm 0.8%), range 0%-2%

^d Number of animals with neoplasm per number of animals with kidney examined microscopically

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

^h Historical incidence for inhalation studies: 2/449 (0.4% \pm 0.9%), range 0%-2%; all routes: 2/1,436 (0.1% \pm 0.5%), range 0%-2%

ⁱ Historical incidence for inhalation studies: 6/449 (1.3% \pm 1.4%), range 0%-4%; all routes: 10/1,436 (0.7% \pm 1.0%), range 0%-4%

^j Historical incidence for inhalation studies: 1/449 (0.2% \pm 0.7%), range 0%-2%; all routes: 2/1,436 (0.1% \pm 0.5%), range 0%-2%

diameter. Microscopically, renal tubule adenomas were typically well circumscribed, discrete, rounded or oval masses greater than five times the diameter of a normal renal tubule, composed of solid aggregates, small nests or tubule-like structures of neoplastic epithelial cells without obvious lumens. The neoplastic cells were round to polygonal in shape with pale-staining, foamy cytoplasm. Larger adenomas had areas of cells with central vacuolation. Seven of the renal tubule cell carcinomas were observed at necropsy and were described as masses or nodules varying in size from 2 mm to 1.5 cm in diameter or as a dilated, thickened pelvis. Microscopically, renal tubule carcinomas were large, less discrete masses of neoplastic cells that were locally invasive and composed of a mixture of round cells with large vesicular nuclei and abundant, pale eosinophilic cytoplasm forming sheets and large nests resembling tubules. Some carcinomas had small numbers of vacuolated, signet ring, neoplastic cells. A few carcinomas had areas of necrosis or tubular structures with necrotic centers surrounded by a thin fibrous stroma. Cellular atypia and pleomorphism were usually present.

One chamber control and one 1,000 ppm male had a lipoma, a benign tumor (Tables 13 and A1). The lipoma in the chamber control male occupied the majority of the renal medulla and was composed of sheets of vacuolated cells with a few residual renal tubules and cystic spaces within the tumor. The lipoma in the 1,000 ppm male also was primarily composed of vacuolated cells but was located in the cortex. These lipomas are infrequent tumors in F344/N rats and unrelated to exposure. The tumor in the chamber control male is the only lipoma in the current historical control data for inhalation studies, and only one other lipoma is included for all routes of exposure combined (Tables 13 and A3b).

Incidences of renal tubule hyperplasia were increased in all exposed groups of male rats and were significantly increased in the 500 and 1,000 ppm groups (Tables 13 and A4). Microscopically, renal tubular cell hyperplasia consisted of mild to marked foci having single to multiple cortical tubules composed of multiple layers of epithelial cells with pale-staining, foamy cytoplasm that partially filled the lumen and enlarged the tubules. The epithelial cells were variably enlarged with distinct cell borders, expanded eosinophilic cytoplasm, variable nuclear size, and multiple, enlarged nucleoli. Affected tubules were generally, but not always, larger than normal but less than five times the diameter of a normal

renal tubule. Renal tubule hyperplasia, as defined in the current study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered a preneoplastic lesion. Renal tubule hyperplasia, adenoma, and carcinoma are part of a morphologic continuum.

The incidences of mineralization of the renal papilla were significantly increased in all exposed groups of males (Tables 13 and A4). Microscopically, the mineralization consisted of elongated profiles of dark basophilic or black granular material within the lumens of the tubules. This linear pattern is consistent with mineralization associated with α_2 u-globulin nephropathy.

Significantly increased incidences of minimal to marked hyperplasia of the transitional epithelium of the renal pelvis occurred in 500 and 1,000 ppm males (Tables 13 and A4). Hyperplasia of the transitional epithelium was characterized by increased numbers of cells in the epithelium lining the pelvis, often with formation of papillary projections. These are common findings in rats and often increase with increased severities of nephropathy.

Although the incidences of minimal to marked nephropathy in exposed groups of males and females were not significantly different from those in the chamber controls, the severity of nephropathy generally increased with increasing exposure concentration (Tables 13, A4, and B4).

Testis: The incidence of interstitial cell adenoma, including bilateral, in 1,000 ppm male rats was significantly increased compared to the chamber control group, and there was a positive trend in the incidences among all exposed groups (Tables 14 and A2). The incidence in the 1,000 ppm group exceeded the range for historical chamber controls in inhalation studies but was within the range for historical controls (all routes) (Tables 14 and A3c). There were increased incidences of bilateral interstitial cell adenoma in all exposed groups (Tables 14 and A1).

Interstitial cell hyperplasia and adenoma are common proliferative lesions in F344/N rats and will develop in nearly all male rats of this strain that are allowed to complete their natural life span. Interstitial cell adenomas begin as nodular aggregates of hyperplasia having characteristically large polyhedral cells with abundant finely

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Testis in Male Rats
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Number Examined Microscopically	50	50	50	50
Interstitial Cell, Hyperplasia ^a	12 (1.9) ^b	18 (2.1)	19 (2.2)	9 (3.0)
Bilateral Interstitial Cell, Hyperplasia	0	0	0	1 (2.0)
Interstitial Cell, Adenoma	18	14	13	9
Bilateral, Interstitial Cell, Adenoma	18	24	27	37
Interstitial Cell Adenoma (includes bilateral) ^c				
Overall rate ^d	36/50 (72%)	38/50 (76%)	40/50 (80%)	46/50 (92%)
Adjusted rate ^e	80.0%	84.6%	85.7%	96.1%
Terminal rate ^f	24/26 (92%)	22/23 (96%)	25/27 (93%)	24/24 (100%)
First incidence (days)	558	536	460	541
Poly-3 test ^g	P=0.006	P=0.370	P=0.311	P=0.007

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

^c Historical incidence for 2-year inhalation studies with chamber control groups (mean \pm standard deviation): 345/449 (76.8% \pm 5.9%), range 66%-84%; all routes: 1,242/1,449 (85.5% \pm 8.7%), range 66%-98%

^d Number of animals with neoplasm per number of animals with testis examined microscopically

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

vacuolated foamy or granular eosinophilic cytoplasm. They have central nuclei with a prominent nucleolus and marginated chromatin. Less commonly, areas of hyperplasia have small spindle cells with dark basophilic nuclei, scant cytoplasm, and indistinct cell boundaries. The distinction between hyperplasia and adenoma can be unclear because interstitial cells can form a continuous morphologic spectrum from small foci of normal appearing cells to large masses extending between and causing compression of the surrounding seminiferous tubules with eventual replacement of the entire testis. An aggregate smaller than a seminiferous tubule is focal hyperplasia. Masses of interstitial cells with a diameter equal in size to or larger than a seminiferous tubule with some compression of adjacent tubules are considered adenomas. As a result of the frequent pres-

ence of both lesions, any testis in which an adenoma, a neoplastic change, was present along with hyperplasia of the interstitial cells, only the adenoma was diagnosed. However, when one testis had an adenoma and the contralateral testis had foci of hyperplasia, both diagnoses were recorded.

Incidences of interstitial cell hyperplasia were increased in 250 and 500 ppm males, and the severity was increased in 1,000 ppm males (Tables 14 and A4). Because of the exposure-related increase in incidences of bilateral interstitial cell adenoma, the incidences of interstitial cell hyperplasia are not well reflected in this data. It is uncertain how the increased incidences of interstitial cell adenoma and hyperplasia were related to cumene exposure.

MICE

2-WEEK STUDY

All mice exposed to 4,000 ppm died on day 1; all mice exposed to 2,000 ppm died by the morning of day 2, and four female mice exposed to 1,000 ppm died by day 4 (Table 15). There were no exposure-related changes in mean body weights among survivors in either sex. Mice exposed to 2,000 ppm were severely lethargic after the first exposure. The four female mice exposed to 1,000 ppm that died early exhibited signs of lethargy and ataxia. Ataxia was most apparent during the first few days of exposure in week 1, and the severity of lethargy and ataxia was greater in females.

Compared to the chamber control groups, absolute and relative liver weights were increased in all groups of sur-

viving male mice and the 250 and 500 ppm females (Table G3). Absolute and relative kidney weights were generally increased in both sexes of surviving mice compared to the chamber control groups; the increases were significant in 250 ppm males. No microscopic changes were present in either sex that would account for the increased liver or kidney weights or for the early deaths. Thymus weights were decreased in 1,000 ppm males.

Exposure Concentration Selection Rationale: Based on the mortalities at 2,000 and 4,000 ppm and signs of central nervous system effects, cumene exposure concentrations selected for the 3-month inhalation study in mice were 62.5, 125, 250, 500, and 1,000 ppm.

TABLE 15
Survival and Body Weights of Mice in the 2-Week Inhalation Study of Cumene

Concentration (ppm)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	21.9 ± 0.3	27.0 ± 0.5	5.2 ± 0.4	
250	5/5	22.6 ± 0.8	27.6 ± 0.6	5.0 ± 0.7	102
500	5/5	23.1 ± 0.8	26.7 ± 0.4	3.6 ± 0.6	99
1,000	5/5	23.1 ± 0.5	27.0 ± 0.9	3.9 ± 0.4	100
2,000	0/5 ^c	23.0 ± 0.5	—	—	—
4,000	0/5 ^d	22.7 ± 0.6	—	—	—
Female					
0	5/5	18.6 ± 0.4	22.5 ± 0.3	3.9 ± 0.2	
250	5/5	19.2 ± 0.6	22.6 ± 0.7	3.5 ± 0.4	100
500	5/5	19.3 ± 0.2	23.3 ± 0.6	4.0 ± 0.8	103
1,000	1/5 ^e	18.9 ± 0.4	23.8	3.6	106
2,000	0/5 ^c	18.9 ± 0.4	—	—	—
4,000	0/5 ^d	18.7 ± 0.7	—	—	—

^a Number of animals surviving at 2 weeks/number initially in group

^b Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the chamber control group are not significant by Dunnett's test.

^c Day of deaths: 2

^d Day of deaths: 1

^e Days of deaths: 3, 3, 3, 4

3-MONTH STUDY

With the exception of eight 1,000 ppm females, all mice survived to the end of the study (Table 16). Final mean body weights and body weight gains of males exposed to 250 ppm or greater were generally less than those of the chamber controls. Ataxia was observed for several days in 1,000 ppm males, but the animals recovered and appeared to develop a tolerance by day 3. The eight 1,000 ppm female mice that died during the first week of the study exhibited clinical signs of acute toxicity, including lethargy or ataxia.

There were no toxicologically relevant changes in the hematology variables for mice (Table F2).

Significant increases in absolute liver weights occurred in mice exposed to 500 or 1,000 ppm, and significant

increases in relative liver weights occurred in groups exposed to 125 ppm or greater (Tables 17 and G4). Although the weight of the cauda epididymis and the spermatid count were significantly decreased in 1,000 ppm males, there were no other significant differences between exposed and chamber control groups in reproductive tissue evaluations in males or vaginal cytology parameters in females (Tables H3 and H4). No exposure-related gross lesions were observed.

Incidences of minimal to mild liver necrosis were significantly increased in male mice exposed to 1,000 ppm (Table 18). Incidences of focal chronic inflammation were significantly increased in females exposed to 62.5, 125, 250, or 500 ppm. Necrosis varied from tiny foci of lymphocytes with a few deeply eosinophilic individual

TABLE 16
Survival and Body Weights of Mice in the 3-Month Inhalation Study of Cumene

Concentration (ppm)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.4 ± 0.3	37.5 ± 0.6	14.1 ± 0.7	
62.5	10/10	23.1 ± 0.3	36.1 ± 0.8	13.0 ± 0.7	96
125	10/10	23.2 ± 0.5	35.8 ± 0.8	12.6 ± 0.8	96
250	10/10	22.9 ± 0.2	35.3 ± 0.7*	12.4 ± 0.6	94
500	10/10	22.9 ± 0.3	34.5 ± 0.8**	11.6 ± 0.8*	92
1,000	10/10	23.2 ± 0.3	34.1 ± 0.6**	10.9 ± 0.7**	91
Female					
0	10/10	19.3 ± 0.3	30.6 ± 1.0	11.3 ± 0.8	
62.5	10/10	19.0 ± 0.2	29.7 ± 1.0	10.7 ± 0.9	97
125	10/10	19.5 ± 0.2	29.6 ± 0.8	10.1 ± 0.8	97
250	10/10	19.3 ± 0.3	29.6 ± 0.8	10.3 ± 0.9	97
500	10/10	19.3 ± 0.3	28.3 ± 0.5	9.0 ± 0.3	92
1,000	2/10 ^c	19.1 ± 0.3	29.2 ± 0.4	8.9 ± 1.1	95

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test

** $P \leq 0.01$

^a Number of animals surviving at 3 months/number initially in group

^b Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

^c Week of deaths: 1

TABLE 17
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice
in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	38.3 ± 0.7	37.7 ± 0.9	37.0 ± 0.8	36.1 ± 0.8	35.8 ± 0.9*	34.7 ± 0.6**
Liver						
Absolute	1.531 ± 0.027	1.601 ± 0.049	1.607 ± 0.041	1.591 ± 0.048	1.705 ± 0.048*	1.913 ± 0.070**
Relative	40.048 ± 0.559	42.490 ± 0.894	43.485 ± 0.551*	44.052 ± 0.746**	47.668 ± 0.795**	55.103 ± 1.501**
Female						
n	10	10	10	10	10	2
Necropsy body wt	32.4 ± 1.1	31.0 ± 1.2	31.4 ± 1.1	31.5 ± 1.1	29.8 ± 0.7	30.8 ± 1.3
Liver						
Absolute	1.453 ± 0.037	1.430 ± 0.047	1.495 ± 0.053	1.552 ± 0.045	1.593 ± 0.042*	1.910 ± 0.110**
Relative	45.016 ± 1.043	46.200 ± 0.778	47.622 ± 0.576*	49.380 ± 0.521**	53.510 ± 0.663**	62.071 ± 1.054**

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

necrotic hepatocytes to foci having coagulative or liquefactive necrosis with scattered neutrophils and lymphocytes involving hepatocytes and adjacent liver tissues. Chronic inflammation consisted of lesions where the predominant features were clusters of lymphocytes and a few neutrophils. Liver necrosis and focal chronic inflammation in male and female mice were considered to be parts of a pathologic continuum.

Sporadic incidences of minimal to mild squamous epithelial hyperplasia and mild inflammation of the mucosa occurred in the forestomach of exposed groups of female mice (Table 18). Squamous epithelial hyperplasia consisted of minimal focal to diffuse thickening of the squamous epithelium. The affected epithelium was five to six cell layers thick as compared to the three to four cell layers for normal squamous epithelium. Acute and chronic inflammation of the forestomach was associated

with hyperplasia in 500 and 1,000 ppm female mice. This inflammation was minimal, was confined to the lamina propria, and consisted of a mixture of inflammatory cells, primarily neutrophils and macrophages. The forestomach lesions had low incidences and were of uncertain relationship to cumene exposure.

Marked necrosis of the thymus was observed in the eight female mice exposed to 1,000 ppm cumene that died during the first week of the study (Table 18). The changes in the thymus were characterized by thymic lymphocytes with small, shrunken, dense nuclei. In multifocal areas, nuclei of the lymphocytes were fragmented or disintegrated. The thymic necrosis was considered to be a nonspecific terminal event associated with glucocorticoid release and not the cause of death; the cause of death was not explained.

TABLE 18
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Male						
Liver ^a	10	10	10	10	10	10
Inflammation, Chronic Focal ^b	4 (1.0) ^c	3 (1.0)	2 (1.0)	3 (1.0)	5 (1.0)	4 (1.0)
Necrosis	0	1 (1.0)	1 (2.0)	1 (1.0)	1 (1.0)	5* (1.2)
Female						
Liver	10	10	10	10	10	10
Inflammation, Chronic Focal	1 (1.0)	10** (1.0)	10** (1.0)	9** (1.0)	7** (1.0)	2 (1.0)
Necrosis	4 (1.3)	0	0	0	2 (1.5)	0
Forestomach	10	10	10	10	10	10
Hyperplasia, Squamous	0	1 (1.0)	0	0	2 (2.0)	1 (1.0)
Inflammation, Acute	0	0	0	0	0	1 (1.0)
Inflammation, Chronic Active	0	0	0	2 (1.0)	2 (1.0)	0
Thymus	10	10	10	10	10	10
Necrosis	0	0	0	0	0	8** (4.0)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test

** $P \leq 0.01$

^a Number of animals with tissue examined microscopically

^b Number of animals with lesion

^c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Exposure Concentration Selection Rationale: Based on slight decreases in body weights in males and minimal effects on organ weights and incidences of lesions, cumene exposure concentrations selected for the 2-year inhalation study in male mice were 250, 500, and 1,000 ppm. Due to the lower survival rate

for 1,000 ppm females, incidences of thymic necrosis at 1,000 ppm, and incidences of liver and forestomach lesions, cumene exposure concentrations selected for the 2-year inhalation study in female mice were 125, 250, and 500 ppm.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 19 and in the Kaplan-Meier survival curves (Figure 4). An exposure concentration-related decrease in survival occurred in male mice, and the survival of 1,000 ppm males was significantly less than that of the chamber controls. Survival of exposed groups of female mice was similar to that of the chamber controls.

Body Weights and Clinical Findings

Mean body weights of 1,000 ppm males were generally less than those of the chamber controls after week 8 of the study, and those of 500 ppm females were less from week 28 until week 76 of the study (Figure 5; Tables 20 and 21). There were no clinical findings related to exposure to cumene; however, thinness and abnormal breathing were observed somewhat more frequently in 1,000 ppm males and 500 ppm females late in the study.

TABLE 19
Survival of Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	7	9	13	20
Natural deaths	5	7	7	7
Animals surviving to study termination	38	34	30	23
Percent probability of survival at end of study ^a	76	68	60	46
Mean survival (days) ^b	709	696	693	665
Survival analysis ^c	P=0.001	P=0.475	P=0.150	P=0.004
	Chamber Control	125 ppm	250 ppm	500 ppm
Female				
Animals initially in study	50	50	50	50
Moribund	8	10	8	12
Natural deaths	5	4	3	3
Animals surviving to study termination	37	36	39	35 ^d
Percent probability of survival at end of study	74	72	78	70
Mean survival (days)	685	698	712	714
Survival analysis	P=0.996	P=1.000	P=0.728N	P=0.982

^a Kaplan-Meier determinations

^b Mean of all deaths (uncensored, censored, and terminal sacrifice)

^c The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A lower mortality in an exposed group is indicated by N.

^d Includes one animal that died during the last week of the study

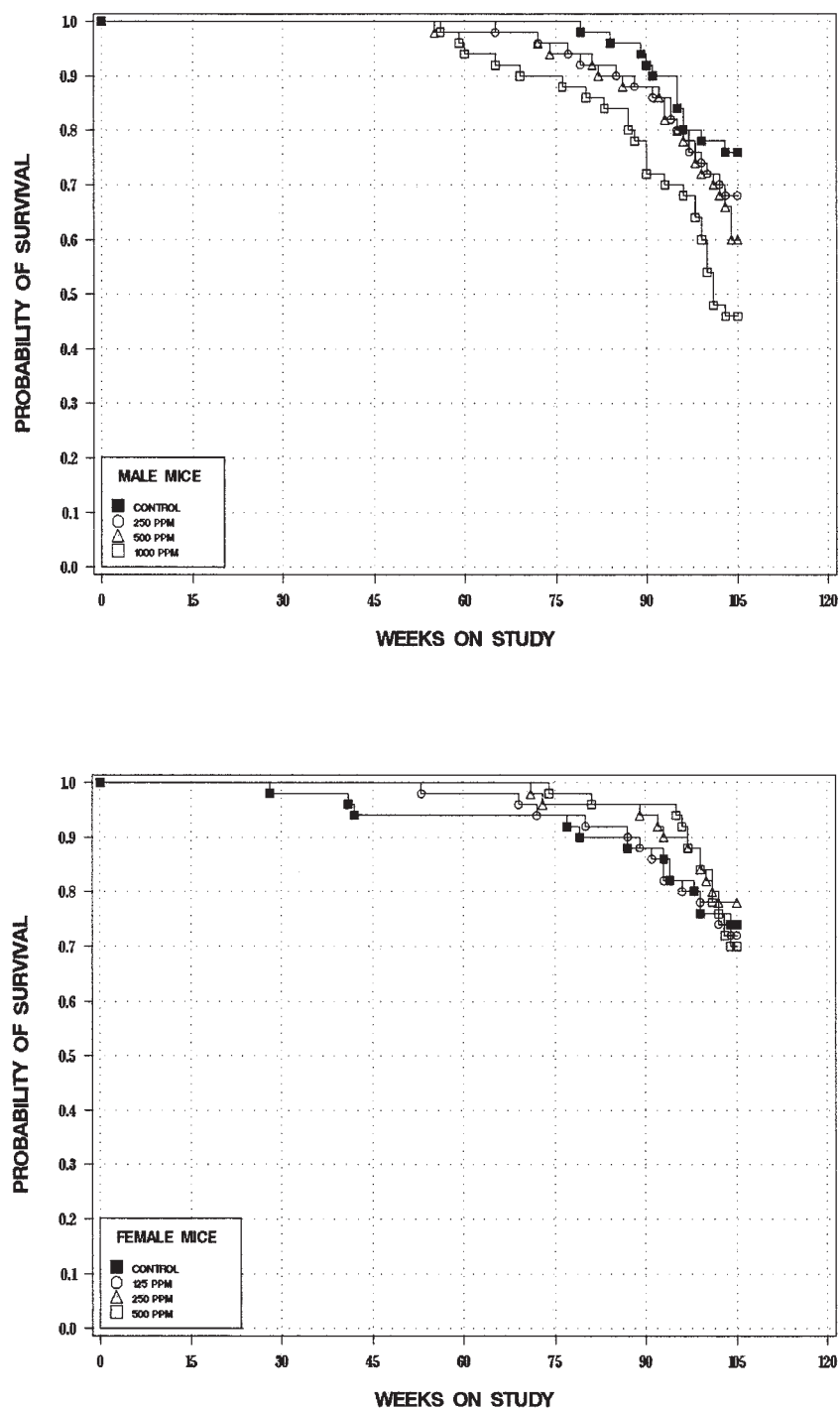


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice
Exposed to Cumene by Inhalation for 2 Years

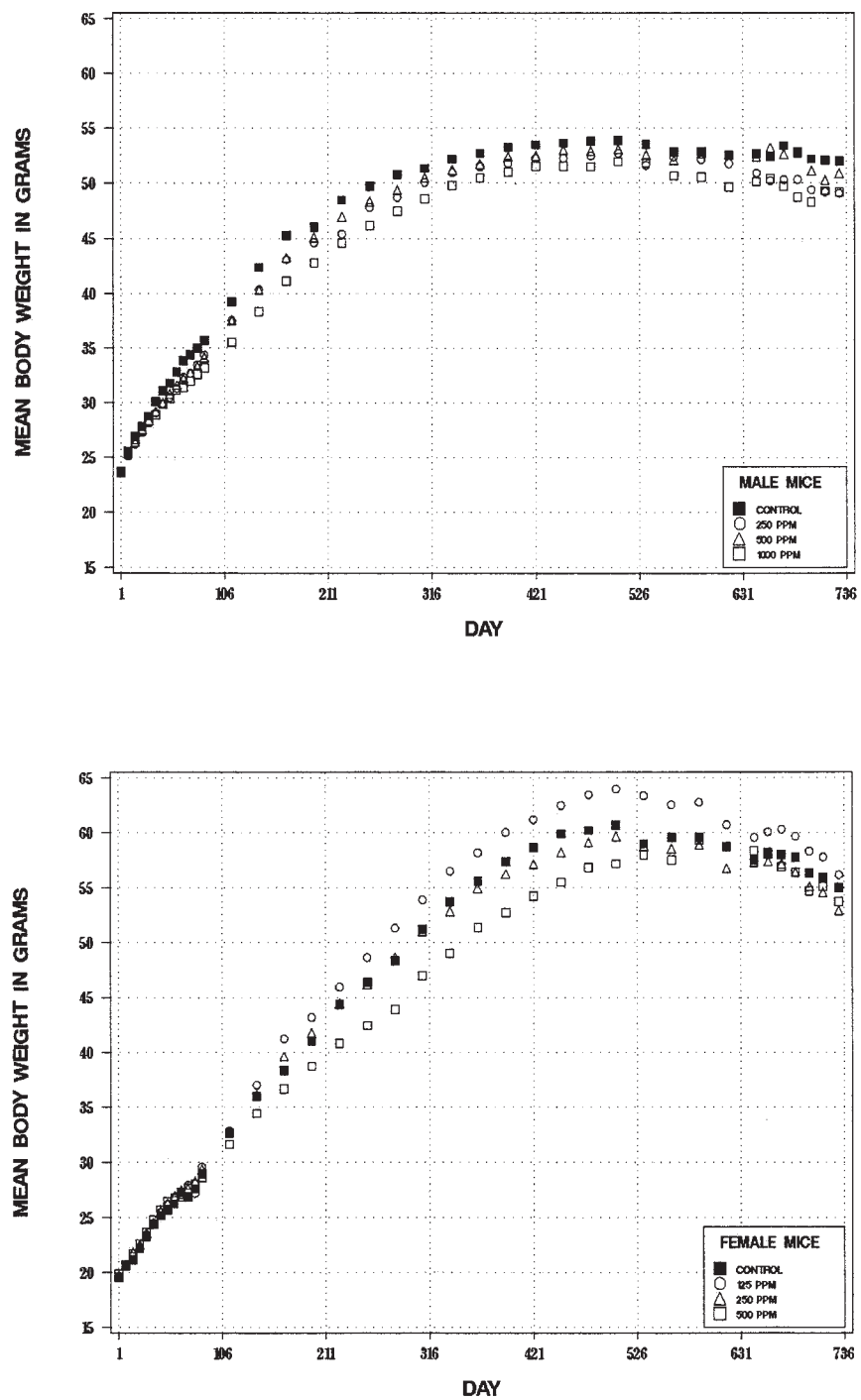


FIGURE 5
Growth Curves for Male and Female Mice
Exposed to Cumene by Inhalation for 2 Years

TABLE 20
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Cumene

Days on Study	Chamber Control		250 ppm			500 ppm			1,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.7	50	23.8	100	50	23.7	100	50	23.6	99	50
8	25.6	50	25.1	98	50	25.5	100	50	25.3	99	50
15	26.9	50	26.2	97	50	26.4	98	50	26.6	99	50
22	27.8	50	27.2	98	50	27.5	99	50	27.3	98	50
29	28.7	50	28.1	98	50	28.4	99	50	28.3	98	50
36	30.1	50	29.0	97	50	29.2	97	50	28.9	96	50
43	31.1	50	29.9	96	50	30.0	96	50	29.8	96	50
50	31.8	50	30.5	96	50	30.8	97	50	30.3	96	50
57	32.8	50	31.3	95	50	31.6	96	50	31.1	95	50
64	33.8	50	32.3	95	50	32.2	95	50	31.4	93	50
71	34.4	50	32.7	95	50	32.7	95	50	32.0	93	50
78	35.0	50	33.4	96	50	33.4	95	50	32.6	93	50
85	35.6	50	34.3	96	50	34.1	96	50	33.2	93	50
113	39.2	50	37.5	96	50	37.5	96	50	35.5	91	50
141	42.4	50	40.3	95	50	40.3	95	50	38.3	90	50
169	45.3	50	43.1	95	50	43.2	96	50	41.1	91	50
197	46.0	50	44.6	97	50	45.1	98	50	42.8	93	50
225	48.5	50	45.4	94	50	47.0	97	50	44.6	92	50
253	49.7	50	47.8	96	50	48.3	97	50	46.2	93	50
281	50.8	50	48.7	96	50	49.4	97	50	47.5	94	50
309	51.4	50	50.1	98	50	50.5	98	50	48.6	95	50
337	52.2	50	51.0	98	50	51.2	98	50	49.8	95	50
365	52.7	50	51.5	98	50	51.7	98	50	50.5	96	50
393	53.2	50	51.8	97	50	52.4	98	49	51.0	96	49
421	53.5	50	52.2	98	50	52.5	98	49	51.5	96	47
449	53.6	50	52.3	98	50	53.0	99	49	51.6	96	47
477	53.8	50	52.5	98	49	53.0	98	49	51.5	96	46
505	53.9	50	52.7	98	48	53.1	99	48	52.0	96	45
533	53.5	50	51.6	96	48	52.6	98	47	51.8	97	44
561	52.8	49	52.4	99	46	52.1	99	47	50.7	96	43
589	52.8	48	52.1	99	46	52.7	100	45	50.6	96	42
617	52.5	48	51.8	99	44	52.5	100	44	49.6	95	39
645	52.7	45	50.9	97	43	52.4	100	43	50.1	95	36
659	52.4	45	50.2	96	41	53.2	102	41	50.4	96	35
673	53.4	40	50.3	94	40	52.6	99	39	49.7	93	34
687	52.8	40	50.3	95	38	52.7	100	37	48.7	92	32
701	52.2	39	49.4	95	36	51.1	98	36	48.3	93	27
715	52.1	39	49.2	95	35	50.3	97	34	49.3	95	24
Mean for weeks											
1-13	30.6		29.5	96		29.7	97		29.3	96	
14-52	47.3		45.4	96		45.8	97		43.8	93	
53-103	53.0		51.3	97		52.4	99		50.5	95	

TABLE 21
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Cumene

Days on Study	Chamber Control		125 ppm			250 ppm			500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.6	50	19.7	100	50	19.8	101	50	19.8	101	50
8	20.7	50	20.6	99	50	20.6	99	50	20.7	100	50
15	21.2	50	21.5	101	50	21.9	103	50	21.7	103	50
22	22.2	50	22.3	101	50	22.5	102	50	22.6	102	50
29	23.3	50	23.4	101	50	23.6	101	50	23.7	102	50
36	24.4	50	24.5	100	50	24.6	101	50	24.8	102	50
43	25.2	50	25.5	101	50	25.6	101	50	25.7	102	50
50	25.7	50	26.2	102	50	26.3	102	50	26.5	103	50
57	26.3	50	26.6	101	50	27.0	103	50	26.8	102	50
64	27.3	50	27.1	99	50	27.5	101	50	26.9	99	50
71	26.9	50	27.9	104	50	27.7	103	50	27.6	103	50
78	27.6	50	27.2	99	50	28.3	103	50	28.1	102	50
85	28.9	50	29.6	102	50	29.4	102	50	28.6	99	50
113	32.7	50	32.9	101	50	32.7	100	50	31.6	97	50
141	36.0	50	37.0	103	50	36.2	101	50	34.4	96	50
169	38.3	50	41.2	108	50	39.6	103	50	36.7	96	50
197	41.0	49	43.2	105	50	41.7	102	50	38.7	94	50
225	44.4	49	46.0	104	50	44.5	100	50	40.8	92	50
253	46.4	49	48.7	105	50	46.2	100	50	42.4	92	50
281	48.3	49	51.3	106	50	48.7	101	50	43.9	91	50
309	51.3	47	53.9	105	50	51.0	100	50	47.0	92	50
337	53.7	47	56.5	105	50	52.9	98	50	49.0	91	50
365	55.6	47	58.2	105	50	55.0	99	50	51.4	92	50
393	57.3	47	60.0	105	49	56.2	98	50	52.7	92	50
421	58.6	47	61.2	104	49	57.1	97	50	54.3	93	50
449	59.9	47	62.5	104	49	58.2	97	50	55.5	93	50
477	60.2	47	63.5	105	49	59.1	98	50	56.8	94	50
505	60.7	47	64.0	106	47	59.7	98	49	57.2	94	50
533	59.0	47	63.4	107	47	58.7	100	48	58.0	98	49
561	59.6	45	62.5	105	46	58.5	98	48	57.5	97	49
589	59.6	45	62.7	105	46	58.9	99	48	59.3	100	48
617	58.7	44	60.7	103	45	56.7	97	48	58.7	100	48
645	57.5	44	59.6	104	43	57.3	100	46	58.4	102	48
659	58.1	41	60.1	104	41	57.4	99	45	58.2	100	48
673	58.0	41	60.3	104	40	57.2	99	45	56.9	98	46
687	57.8	40	59.7	103	40	56.4	98	43	56.4	98	44
701	56.3	38	58.3	104	39	55.1	98	41	54.7	97	42
715	56.0	38	57.8	103	37	54.6	98	39	55.1	98	38
Mean for weeks											
1-13	24.6		24.8	101		25.0	102		24.9	101	
14-52	43.6		45.6	105		43.7	100		40.5	93	
53-103	58.3		60.9	104		57.3	98		56.3	97	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung, nose, liver, forestomach, spleen, and thyroid gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Lung: The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) in all exposed groups of mice occurred with positive trends, were significantly greater than those in the chamber controls, and exceeded the ranges for historical chamber controls in inhalation studies and historical controls (all routes) (Tables 22, C2, C3a, D2, and D3a). Significantly increased incidences of multiple alveolar/bronchiolar adenoma and multiple alveolar/bronchiolar carcinoma occurred in all exposed groups. Microscopically, most of the alveolar/bronchiolar adenomas had papillary patterns (Plates 8 and 9). Tumor margins were usually well-demarcated with compression of the surrounding parenchyma. Alveolar/bronchiolar carcinomas varied from well-differentiated neoplasms with papillary patterns to poorly circumscribed, infiltrative tumors consisting of densely packed pleomorphic cells having multiple layers of nuclei (Plates 10 and 11). Often, prominent alveolar infiltrates of macrophages and occasional multinucleate giant cells were associated with these carcinomas.

The incidences of alveolar epithelial bronchiole metaplasia and bronchiole hyperplasia were significantly increased in all exposed groups of mice, and the severity increased in all exposed groups (Tables 22, C4, and D4). Microscopically, alveolar epithelial bronchiole metaplasia occurs when the flat epithelium of the alveolar ducts and adjacent alveolar septa is replaced by cells similar in appearance to those normally lining terminal bronchioles. Minimal to marked alveolar epithelial bronchiole metaplasia in the exposed mice was characterized by increases in the numbers of plump cuboidal epithelial cells having large hyperchromatic nuclei that lined the alveolar walls adjacent to and extending from the terminal bronchioles (Plates 12 and 13). Minimal to marked bronchiole hyperplasia resulted in papillary projections into the bronchiolar lumens by cuboidal epithe-

lial cells lining the bronchioles (Plate 14). When these lesions had marked hyperplasia, the epithelium filled the bronchi and resembled early alveolar/bronchiolar adenomas.

Nose: Exposure to cumene resulted in increased incidences of olfactory epithelium atrophy, olfactory epithelium basal cell hyperplasia, olfactory epithelium atypical hyperplasia, olfactory epithelium glands hyperplasia, respiratory epithelium squamous metaplasia, and suppurative inflammation (Tables 23, C4, and D4). Although nasal lesions were seen in all three nasal cavity levels that are routinely examined in NTP toxicity and carcinogenicity studies, they predominantly occurred in Level III and to a lesser extent in Level I.

The incidences of olfactory epithelium atrophy in all exposed groups of males and 125 and 500 ppm females were significantly greater than those in the chamber controls (Tables 23, C4, and D4). Microscopically, minimal to mild olfactory epithelium atrophy consisted of small focal lesions involving primarily the epithelium lining the dorsal meatus in Level III nasal sections and occasionally the ethmoid turbinates. The epithelium was thin, with decreased numbers of olfactory neurons (Plate 15); often, metaplasia of the olfactory epithelium to ciliated columnar epithelium and diffuse loss of Bowman's glands in the adjacent lamina propria occurred.

The incidences of basal cell hyperplasia of the olfactory epithelium were significantly increased in 500 and 1,000 ppm males and 250 and 500 ppm females (Tables 23, C4, and D4). Minimal to moderate basal cell hyperplasia of the olfactory epithelium occurred in Level III nasal sections. Minimal hyperplasia was characterized by increased numbers of basal cells crowded along the basement membrane of the olfactory epithelium. In addition to increased basal cells along the basement membrane, mild lesions had infiltration of the adjacent lamina propria by basal cells, with occasional formation of small rosettes within the epithelium. In moderate lesions, the increase in basal cells was accompanied by disruption of the olfactory epithelium.

The incidences of atypical hyperplasia of the olfactory epithelium were significantly increased in groups of mice exposed to 500 ppm or greater (Tables 23, C4, and D4). Minimal to moderate atypical hyperplasia of the olfactory epithelium consisted of proliferation of atypical basal cells with disruption and infiltration of the adjacent lamina propria of ethmoid turbinates

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Bronchiole, Metaplasia ^a	5 (1.4) ^b	43** (2.9)	42** (3.1)	39** (3.0)
Bronchiole, Hyperplasia	0	11** (2.1)	17** (3.2)	18** (2.8)
Alveolar/bronchiolar Adenoma, Multiple	1	12**	15**	20**
Alveolar/bronchiolar Adenoma (includes multiple) ^c				
Overall rate ^d	13/50 (26%)	31/50 (62%)	31/50 (62%)	29/50 (58%)
Adjusted rate ^e	27.5%	66.7%	66.9%	67.9%
Terminal rate ^f	10/38 (26%)	25/34 (74%)	23/30 (77%)	20/23 (87%)
First incidence (days)	628	551	512	480
Poly-3 test ^g	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Carcinoma, Multiple	0	8**	20**	17**
Alveolar/bronchiolar Carcinoma (includes multiple) ^h				
Overall rate	9/50 (18%)	19/50 (38%)	32/50 (64%)	33/50 (66%)
Adjusted rate	19.1%	41.5%	70.5%	71.3%
Terminal rate	6/38 (16%)	15/34 (44%)	25/30 (83%)	12/23 (52%)
First incidence (days)	631	551	565	420
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma ⁱ				
Overall rate	19/50 (38%)	38/50 (76%)	42/50 (84%)	43/50 (86%)
Adjusted rate	39.8%	81.4%	89.5%	92.1%
Terminal rate	14/38 (37%)	31/34 (91%)	30/30 (100%)	21/23 (91%)
First incidence (days)	628	551	512	420
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
	Chamber Control	125 ppm	250 ppm	500 ppm
Female				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Bronchiole, Metaplasia	0	42** (2.6)	49** (2.9)	47** (3.3)
Bronchiole, Hyperplasia	0	17** (2.7)	10** (2.8)	14** (2.8)
Alveolar/bronchiolar Adenoma, Multiple	0	13**	20**	30**
Alveolar/bronchiolar Adenoma (includes multiple) ^j				
Overall rate	1/50 (2%)	26/50 (52%)	36/50 (72%)	38/50 (76%)
Adjusted rate	2.3%	56.3%	74.5%	77.9%
Terminal rate	1/37 (3%)	21/36 (58%)	31/39 (80%)	29/35 (83%)
First incidence (days)	731 (T)	555	495	565
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Female (continued)				
Alveolar/bronchiolar Carcinoma, Multiple	0	6*	7**	19**
Alveolar/bronchiolar Carcinoma (includes multiple) ^k				
Overall rate	3/50 (6%)	16/50 (32%)	20/50 (40%)	34/50 (68%)
Adjusted rate	6.7%	35.3%	41.9%	69.5%
Terminal rate	2/37 (5%)	13/36 (36%)	15/39 (39%)	24/35 (69%)
First incidence (days)	533	646	618	513
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma ^l				
Overall rate	4/50 (8%)	31/50 (62%)	42/50 (84%)	46/50 (92%)
Adjusted rate	8.9%	66.8%	86.0%	92.4%
Terminal rate	3/37 (8%)	25/36 (69%)	34/39 (87%)	33/35 (94%)
First incidence (days)	533	555	495	513
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

(T) Terminal sacrifice

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

^c Historical incidence for 2-year inhalation studies with chamber control groups (mean \pm standard deviation): 92/449 (20.5% \pm 5.3%), range 12%-26%; all routes: 263/1,498 (17.9% \pm 6.1%), range 6%-28%

^d Number of animals with neoplasm per number of animals with lung examined microscopically

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

^h Historical incidence for inhalation studies: 64/449 (14.2% \pm 4.6%), range 10%-24%; all routes: 161/1,498 (10.9% \pm 5.6%), range 2%-24%

ⁱ Historical incidence for inhalation studies: 146/449 (32.5% \pm 5.9%), range 26%-44%; all routes: 401/1,498 (27.2% \pm 7.8%), range 12%-44%

^j Historical incidence for inhalation studies: 19/449 (4.2% \pm 2.5%), range 2%-8%; all routes: 77/1,596 (4.9% \pm 2.7%), range 0%-12%

^k Historical incidence for inhalation studies: 15/449 (3.4% \pm 3.9%), range 0%-12%; all routes: 57/1,596 (3.6% \pm 3.1%), range 0%-12%

^l Historical incidence for inhalation studies: 34/449 (7.6% \pm 4.0%), range 2%-14%; all routes: 129/1,596 (8.2% \pm 3.9%), range 2%-18%

by irregular polyhedral cells. These cells had hyperchromatic, round to oval nuclei and scant basophilic cytoplasm. Frequently, rosettes of columnar cells with basally located nuclei around a central lumen were formed. Moderate atypical basal cell hyperplasia in four 1,000 ppm male mice had features of preneoplastic lesions (Plate 16).

The incidences of hyperplasia of olfactory epithelium glands were significantly increased in all exposed groups of males and in 500 ppm females (Tables 23, C4,

and D4). Minimal focal hyperplasia of olfactory epithelium glands consisted of a few dilated Bowman's glands lined by multiple layers of epithelial cells.

The incidence of squamous metaplasia of the respiratory epithelium was significantly increased in 500 ppm females (Tables 23 and D4). Minimal to mild squamous metaplasia of the respiratory epithelium was characterized by replacement of the normal cuboidal to columnar epithelium along the tips of the turbinates in nasal section Level I by squamous epithelial cells.

TABLE 23
Incidences of Selected Nonneoplastic Lesions of the Nose in Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	49	48
Olfactory Epithelium, Atrophy ^a	4 (1.3) ^b	13* (1.1)	11* (1.2)	38** (1.4)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	15** (1.0)	33** (1.1)
Olfactory Epithelium, Hyperplasia, Atypical	0	0	5* (1.6)	11** (1.7)
Olfactory Epithelium, Glands, Hyperplasia	3 (1.0)	11* (1.0)	9* (1.1)	23** (1.0)
Inflammation, Suppurative	2 (2.0)	2 (1.5)	9* (1.1)	6 (1.5)
Female				
Number Examined Microscopically	50	50	50	50
Olfactory Epithelium, Atrophy	4 (1.0)	11* (1.1)	9 (1.1)	18** (1.2)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	1 (1.0)	11** (1.0)	25** (1.1)
Olfactory Epithelium, Hyperplasia, Atypical	0	0	2 (1.0)	10** (1.2)
Olfactory Epithelium, Glands, Hyperplasia	1 (1.0)	4 (1.0)	4 (1.0)	11** (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	1 (2.0)	6* (1.2)
Inflammation, Suppurative	0	1 (1.0)	3 (1.3)	7* (1.3)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

The incidences of suppurative inflammation were significantly increased in 500 ppm males and females (Tables 23, C4, and D4). Minimal to mild suppurative inflammation resulted from infiltration of the lamina propria by various inflammatory cells, predominantly neutrophils, and was associated with necrosis or squamous metaplasia of the overlying epithelium.

Liver: In females, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) occurred with positive trends and were significantly increased in the 500 ppm group; incidences of these lesions in all exposed groups of females and the incidence of hepatocellular carcinoma in 500 ppm females exceeded the historical ranges for chamber controls but were generally within the historical ranges for controls (all routes) (Tables 24, D2, and D3b). Microscopically, the hepatocellular adenomas and hepatocellular carcinomas had the typical appearance of these tumors as reported in B6C3F1 mice. Hepatocellular adenomas were usually discrete masses

having solid growth patterns that caused compression of the surrounding normal hepatic parenchyma. They consisted of hepatocytes having clear, eosinophilic, or basophilic cytoplasm, and were sometimes difficult to distinguish from hepatocellular foci. However, the lack of normal lobular architecture and the presence of plates of neoplastic hepatocytes that intersected the surrounding normal liver plates at sharp angles, rather than merging with them as seen in foci, were characteristics used to differentiate adenomas from foci. Hepatocellular carcinomas were large, poorly demarcated masses that generally had irregular borders due to growth into the surrounding normal parenchyma. The neoplastic hepatocytes often were somewhat atypical in appearance, but the major distinguishing feature of carcinomas was the presence of abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly, the neoplastic cells formed glandular structures or solid masses. Several growth patterns were often seen within a single

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus ^a	6	5	16**	14*
Hepatocellular Adenoma, Multiple	17	20	22	26
Hepatocellular Adenoma (includes multiple) ^b	34	33	37	35
Hepatocellular Carcinoma, Multiple	3	1	4	7
Hepatocellular Carcinoma (includes multiple) ^c	13	18	21	17
Hepatocellular Adenoma or Carcinoma ^d				
Overall rate ^e	40/50 (80%)	42/50 (84%)	43/50 (86%)	41/50 (82%)
Adjusted rate ^f	81.0%	85.8%	87.2%	87.1%
Terminal rate ^g	30/38 (79%)	28/34 (82%)	26/30 (87%)	20/23 (87%)
First incidence (days) ^h	551	453	381	391
Poly-3 test ^h	P=0.250	P=0.355	P=0.284	P=0.286
	Chamber Control	125 ppm	250ppm	500 ppm
Female				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus	8	11	7	14
Hepatocellular Adenoma, Multiple	9	13	9	10
Hepatocellular Adenoma (includes multiple) ⁱ				
Overall rate	18/50 (36%)	23/50 (46%)	27/50 (54%) ^j	29/50 (58%)
Adjusted rate	40.5%	50.0%	56.4%	59.8%
Terminal rate	17/37 (46%)	17/36 (47%)	22/39 (56%)	19/35 (54%)
First incidence (days)	654	609	618	662
Poly-3 test	P=0.040	P=0.243	P=0.091	P=0.046
Hepatocellular Carcinoma, Multiple	2	1	2	0
Hepatocellular Carcinoma (includes multiple) ^k	10	7	6	12

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Female (continued)				
Hepatocellular Adenoma or Carcinoma ^l				
Overall rate	25/50 (50%)	26/50 (52%)	29/50 (58%) ^j	36/50 (72%)
Adjusted rate	55.6%	56.5%	60.4%	74.1%
Terminal rate	22/37 (60%)	20/36 (56%)	23/39 (59%)	25/35 (71%)
First incidence (days)	607	609	618	662
Poly-3 test	P=0.024	P=0.549	P=0.395	P=0.043

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test

** $P \leq 0.01$

a Number of animals with lesion

b Historical incidence for 2-year inhalation studies with chamber control groups (mean \pm standard deviation): 196/449 (43.7% \pm 10.9%), range 30%-68%; all routes: 633/1,496 (43.3% \pm 14.2%), range 14%-70%

c Historical incidence for inhalation studies: 107/449 (23.8% \pm 4.6%), range 18%-32%; all routes: 382/1,496 (26.0% \pm 9.1%), range 8%-48%

d Historical incidence for inhalation studies: 264/449 (58.8% \pm 9.6%), range 50%-80%; all routes: 874/1,496 (59.6% \pm 15.4%), range 20%-85%

e Number of animals with neoplasm per number of animals with liver examined microscopically

f Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

g Observed incidence at terminal kill

h Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

i Historical incidence for inhalation studies: 109/447 (24.4% \pm 8.7%), range 12%-36%; all routes: 402/1,593 (25.8% \pm 15.8%), range 2%-62%

j One animal with adenoma also had hepatoblastoma.

k Historical incidence for inhalation studies: 48/447 (10.7% \pm 4.1%), range 6%-20%; all routes: 159/1,593 (10.2% \pm 6.6%), range 0%-28%

l Historical incidence for inhalation studies: 145/447 (32.4% \pm 8.8%), range 22%-50%; all routes: 505/1,593 (32.4% \pm 17.5%), range 8%-64%

neoplasm. Areas of hemorrhage or necrosis were sometimes present. Metastases of carcinomas to the lungs occurred in all groups of mice of both sexes and were often multiple.

The incidences of eosinophilic foci were significantly increased in male mice exposed to 500 or 1,000 ppm (Tables 24 and C4). Eosinophilic foci consisted of well-demarcated collections of enlarged hepatocytes with abundant, dark, homogeneous, eosinophilic cytoplasm. These hepatocytes were arranged in normal hepatic cords that merged with the surrounding normal hepatocytes. Usually, little or no compression of the surrounding normal hepatocytes occurred, although some degree of compression was occasionally evident in larger foci. Eosinophilic foci, hepatocellular adenomas,

and hepatocellular carcinomas are thought to represent a morphologic continuum.

Forestomach: The incidences of epithelial hyperplasia were significantly increased in groups of males exposed to 500 or 1,000 ppm, and the incidences of ulceration and inflammation were significantly increased in the 1,000 ppm group (Tables 25 and C4). These three lesions were often present in the forestomach of the same mouse. Epithelial hyperplasia was characterized by a diffuse thickening of the squamous epithelium due to increased numbers of cell layers, primarily of prickly cells. Ulcers of the forestomach resulted after damage to the mucosal surface, with loss of squamous epithelium extending through the basement membrane. The ulcers were usually accompanied by inflammation. The

TABLE 25
Incidences of Nonneoplastic Lesions of the Forestomach in Male Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Number Examined Microscopically	50	50	50	49
Epithelium, Hyperplasia ^a	2 (2.0) ^b	7 (2.1)	8* (2.3)	13** (2.1)
Ulcer	1 (3.0)	4 (2.8)	6 (2.8)	6* (2.8)
Inflammation	0	2 (2.0)	1 (2.0)	5* (2.0)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

margins of the ulcers often had epithelial hyperplasia. Inflammation of the forestomach had variable numbers of mixed inflammatory cells, with congestion and various degrees of increased fibrous connective tissue.

Spleen: In males, hemangiosarcoma of the spleen occurred with a significant positive trend, and the incidence in the 1,000 ppm group was significantly greater than that in the chamber control group and exceeded the historical ranges for chamber controls in inhalation studies and historical controls (all routes) (Tables 26, C2, and C3c). The incidences of hemangiosarcoma in all organs (heart, liver, urinary bladder, spleen) in male mice also increased with a statistically significant positive trend, and the incidence was significantly increased in 1,000 ppm males. The incidence of hemangiosarcoma in all organs (spleen, liver, mesentery, bone, nose, and skeletal muscle) of 500 ppm females exceeded the historical range for chamber controls in inhalation studies (Tables 26, D2, and D3c). The role of cumene exposure in increased incidences of hemangiosarcoma in male and female mice is uncertain.

Splenic hemangiosarcoma consisted of red and white pulp being replaced with vascular spaces of various sizes lined by plump, pleomorphic endothelial cells and supported by varying amounts of fibrous connective tissue.

Thyroid gland: Incidences of follicular cell adenoma increased with a statistically significant positive trend in males; the incidence in 1,000 ppm males was at the upper end of the historical ranges for chamber controls in inhalation studies and for historical controls (all routes) (Tables 27, C2, and C3d). The incidence of follicular cell hyperplasia was increased in 1,000 ppm males, but the increase was not significant compared to the chamber control group (Tables 27 and C4). These marginal increases were considered possibly related to cumene exposure.

The follicular cell adenomas were well-circumscribed nodules consisting of irregularly shaped follicles containing various amounts of colloid. They were formed by proliferation of hyperchromatic follicular cells and compressed, to various degrees, the adjacent follicles.

TABLE 26
Incidences of Hemangiosarcoma in Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Male				
Hemangiosarcoma, Spleen ^a				
Overall rate ^b	0/50 (0%)	0/50 (0%)	0/49 (0%)	4/50 (8%)
Adjusted rate ^c	0.0%	0.0%	0.0%	9.9%
Terminal rate ^d	0/38 (0%)	0/34 (0%)	0/30 (0%)	3/23 (13%)
First incidence (days)	— ^e	— ^g	—	556
Poly-3 test ^f	P=0.002	—	—	P=0.045
Hemangiosarcoma, All Organs ^h				
Overall rate ⁱ	0/50 (0%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	2.2%	4.5%	9.9%
Terminal rate	0/38 (0%)	0/34 (0%)	2/30 (7%)	3/23 (13%)
First incidence (days)	—	654	729 (T)	556
Poly-3 test	P=0.015	P=0.493	P=0.226	P=0.045
	Chamber Control	125 ppm	250 ppm	500 ppm
Female				
Number Examined Microscopically	49	50	50	50
Hemangiosarcoma, Spleen ^{j,k}	0	0	3	1
Hemangiosarcoma, All Organs ^l				
Overall rate ⁱ	1/50 (2%)	3/50 (6%)	6/50 (12%)	1/50 (2%)
Adjusted rate	2.3%	6.6%	12.8%	2.1%
Terminal rate	1/37 (3%)	1/36 (3%)	5/39 (13%)	1/35 (3%)
First incidence (days)	731 (T)	555	673	731 (T)
Poly-3 test	P=0.518N	P=0.318	P=0.066	P=0.746N

(T) Terminal sacrifice

^a Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 6/444 (1.4% ± 1.5%), range 0%-4%; all routes: 24/1,483 (1.7% ± 1.2%), range 0%-4%

^b Number of animals with neoplasm per number of animals with tissue examined microscopically

^c Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^d Observed incidence at terminal kill

^e Not applicable; no neoplasms in animal group

^f Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by N.

^g Value of statistic cannot be computed.

^h Historical incidence for inhalation studies: 21/450 (4.7% ± 3.7%), range 0%-12%; all routes: 76/1,499 (5.2% ± 3.2%), range 0%-12%

ⁱ Number of animals with neoplasm per number of animals necropsied

^j Number of animals with lesion

^k Historical incidence for inhalation studies: 6/445 (1.3% ± 1.4%), range 0%-4%; all routes: 27/1,573 (1.8% ± 1.8%), range 0%-8%

^l Historical incidence for inhalation studies: 16/449 (3.6 ± 2.2%), range 2%-8%; all routes: 71/1,598 (4.6% ± 3.2%), range 2%-16%

TABLE 27
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Male Mice
in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Number Examined Microscopically	50	50	49	50
Follicular Cell, Hyperplasia ^a	7 (1.9) ^b	7 (2.4)	7 (1.7)	11 (1.9)
Follicular Cell, Adenoma ^c				
Overall rate ^d	0/50 (0%)	0/50 (0%)	0/49 (0%)	3/50 (6%)
Adjusted rate ^e	0.0%	0.0%	0.0%	7.5%
Terminal rate ^f	0/38 (0%)	0/34 (0%)	0/30 (0%)	2/23 (9%)
First incidence (days)	— ^g	— ⁱ	—	680
Poly-3 test ^h	P=0.010	—	—	P=0.095

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

^c Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 5/441 (1.1% ± 2.0%), range 0%-6%; all routes: 21/1,483 (1.4% ± 1.8%), range 0%-6%

^d Number of animals with neoplasm per number of animals with thyroid gland examined microscopically

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g Not applicable; no neoplasms in animal group

^h Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

ⁱ Value of statistic cannot be computed.

GENETIC TOXICOLOGY

Cumene (1 to 333 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 when tested with and without induced rat or hamster liver S9 activation enzymes (Table E1). *In vivo*, cumene induced small, but significant, increases in micronucleated polychromatic erythrocytes in bone marrow of male rats treated by intraperitoneal injection (Table E2). Two trials were performed in rats. In the first trial, doses ranging from 78.13 to 2,500 mg/kg were administered three times at 24-hour intervals, and results were positive, based both on the trend ($P < 0.001$) and the response at the 1,250 mg/kg dose. The data from the 2,500 mg/kg dose were excluded from analysis because only two animals survived and a minimum of three animals is required for a valid dose point. The second confirmatory trial also produced a positive response, although the trend test was not significant ($P = 0.085$). Micronucleated erythrocytes were elevated at all four

doses in trial 2; the responses at the 312 and 1,250 mg/kg levels were statistically significant ($P < 0.006$). Because considerable toxicity was again observed at the high dose of 2,500 mg/kg, the trend was recalculated over the same dose range as for the first trial (0 to 1,250 mg/kg), and a significant P value of 0.019 was produced. The percentage of polychromatic erythrocytes in the bone marrow fluctuated unrelated to dose and likely represented variation within the normal range of 40% to 60% polychromatic erythrocytes among the total erythrocyte population in the bone marrow. In contrast to the results in male rats, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation (62.5 to 1,000 ppm) for 3 months (Table E3). For both male and female mice, no significant changes in the percentage of polychromatic erythrocytes were observed over the exposure range tested, indicating an absence of treatment-related toxicity to the bone marrow.



PLATE 1

Nasal cavity of a female F344/N rat exposed to 250 ppm cumene in the 2-year inhalation study. Adenoma arising from the respiratory epithelium lining the nasoturbinate in nasal Level I. H&E

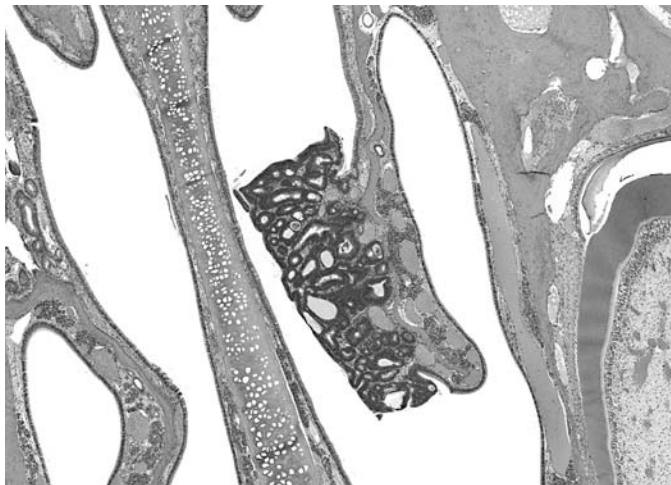


PLATE 2

Nasal cavity of a female F344/N rat exposed to 250 ppm cumene in the 2-year inhalation study. Adenoma arising from the respiratory epithelium lining the nasoturbinate in nasal Level II. Note the formation of acinar patterns by basophilic neoplastic cells. H&E

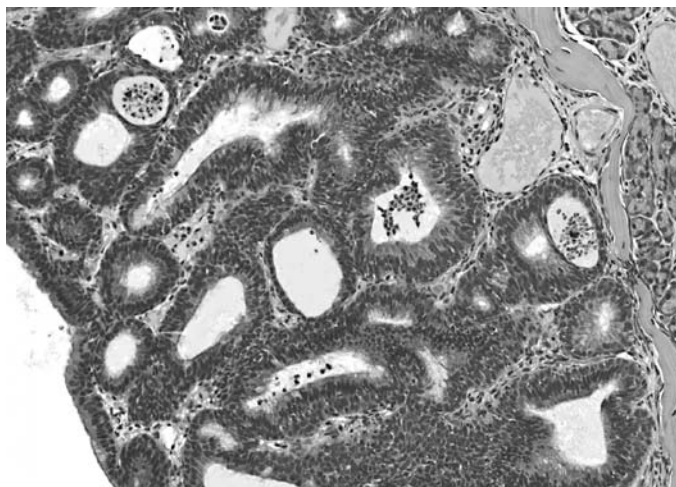


PLATE 3

Higher magnification of Plate 2. Note the acinar patterns formed by cords of neoplastic basophilic respiratory epithelium. H&E

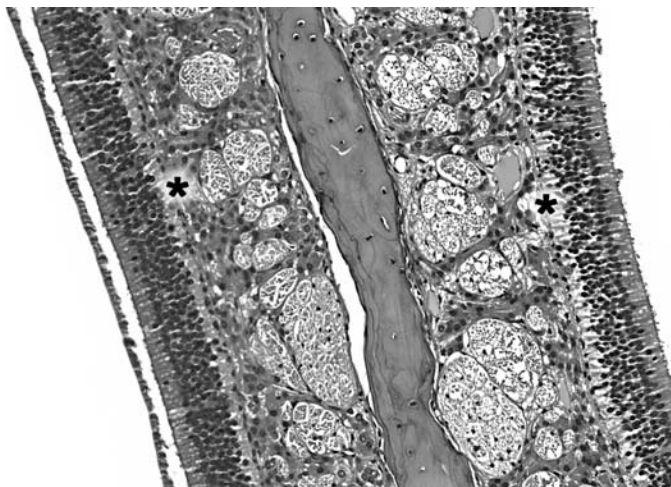


PLATE 4

Olfactory epithelium with a normal, orderly, thin basal cell layer (asterisks) beneath the multiple layers of neuron nuclei in nasal Level III of a male chamber control F344/N rat at 2 years in the inhalation study of cumene. H&E

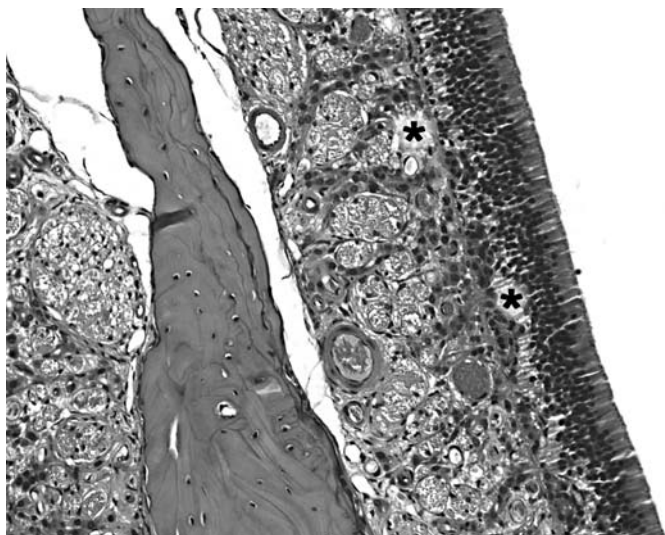


PLATE 5

Olfactory epithelium with minimal basal cell hyperplasia in nasal Level III of a female F344/N rat exposed to 1,000 ppm cumene in the 2-year inhalation study. Note the increased numbers of basophilic cells along the basement membrane (asterisks) under more orderly, normal appearing olfactory neuron nuclei. H&E

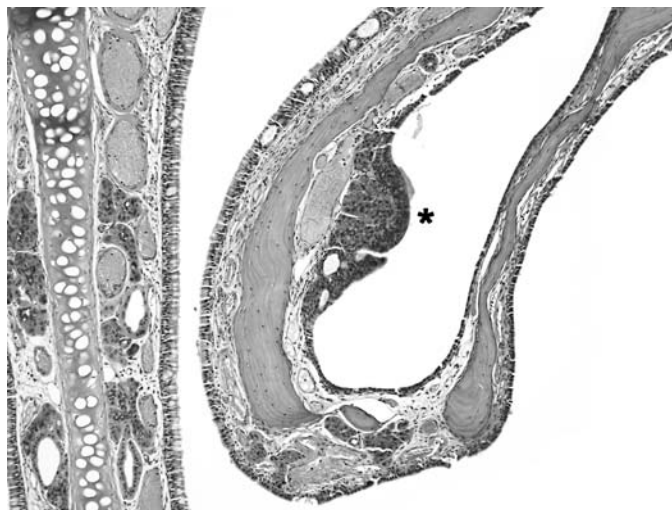


PLATE 6

Nasal cavity of a male F344/N rat exposed to 1,000 ppm cumene in the 2-year inhalation study. Note the localized hyperplasia of the respiratory epithelium lining the nasoturbinate in nasal Level I (asterisk). H&E

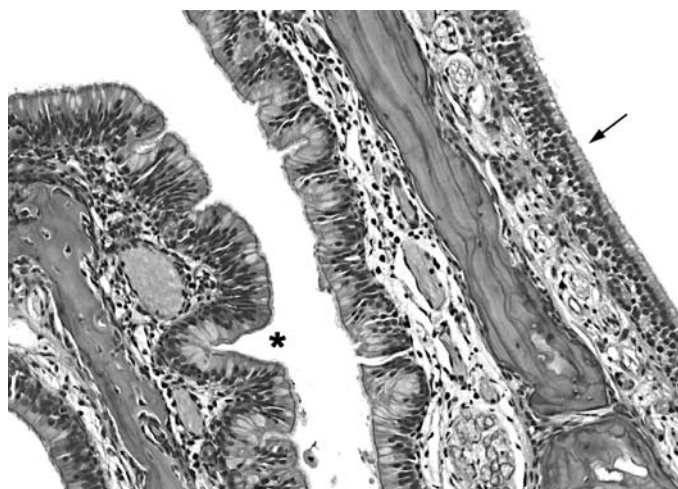


PLATE 7

Olfactory epithelium in nasal Level III of a male F344/N rat exposed to 500 ppm cumene in the 2-year inhalation study. Note the undulating mucosa resulting from goblet cell hyperplasia of metaplastic, respiratory-like epithelial cells (asterisk). Compare these changes with the more normal olfactory epithelium lining the opposite surface of the nasal septum (arrow). H&E

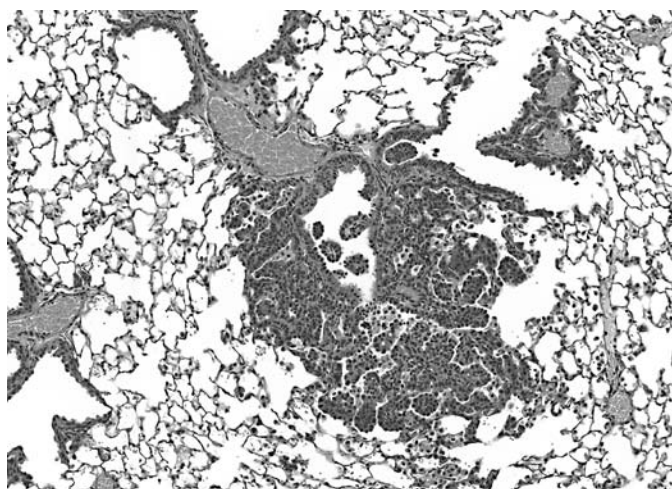


PLATE 8

Lung of a female B6C3F1 mouse exposed to 250 ppm cumene in the 2-year inhalation study. An alveolar/bronchiolar adenoma arising at the alveolar-bronchiolar junction with extension into adjacent alveoli. Note the papillary patterns. H&E

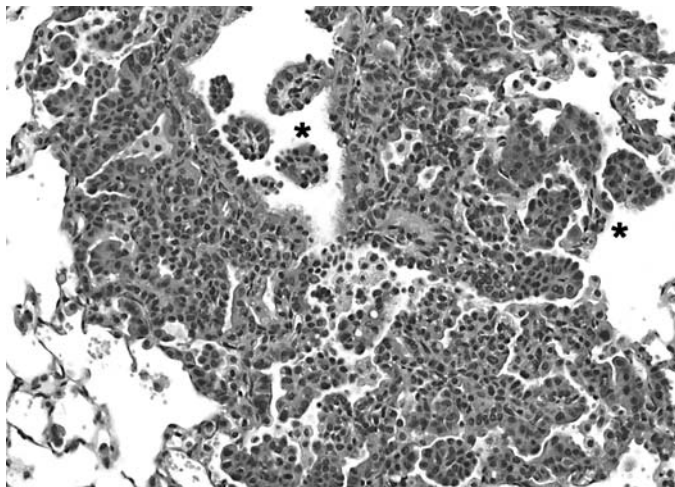


PLATE 9

Higher magnification of Plate 8. Note the papillary patterns formed by cuboidal neoplastic epithelial cells (asterisks). H&E

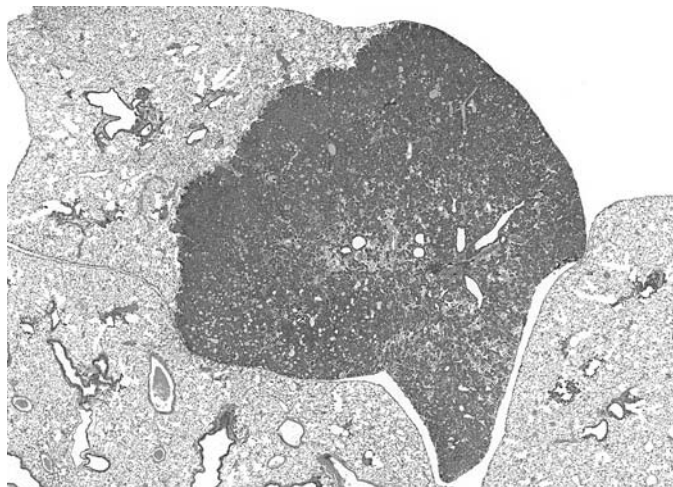


PLATE 10

Lung of a male B6C3F1 mouse exposed to 500 ppm cumene in the 2-year inhalation study. This alveolar/bronchiolar carcinoma involves a large portion of the affected lobe. H&E

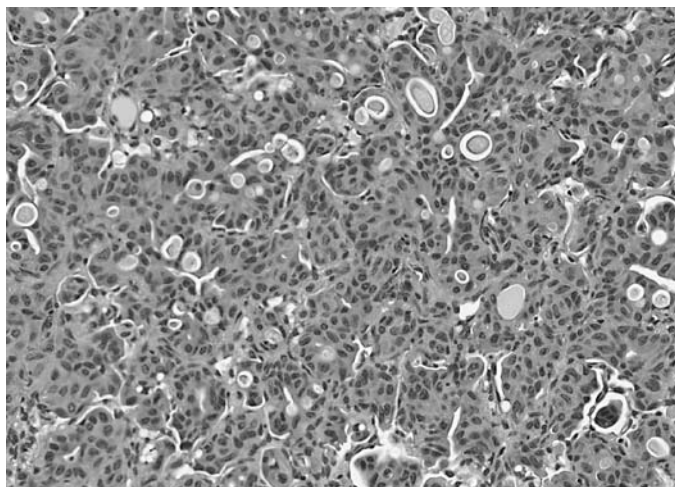


PLATE 11

Higher magnification of Plate 10. Note that although the neoplastic cells are anaplastic and pleomorphic, cuboidal epithelial features persist. H&E

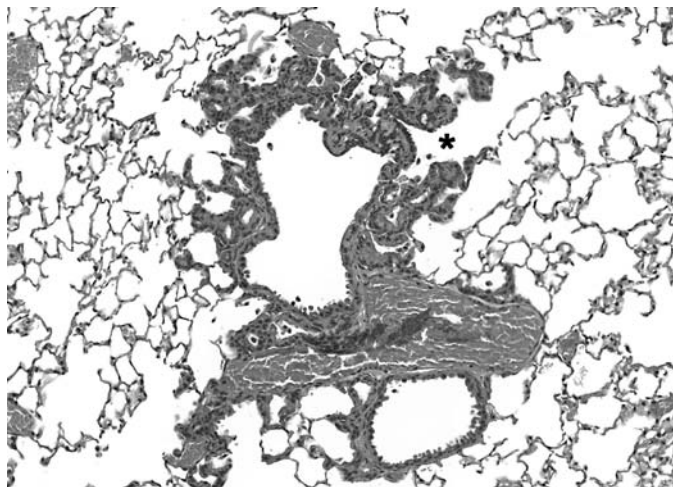


PLATE 12

Lung of a female B6C3F1 mouse exposed to 500 ppm cumene in the 2-year inhalation study. Note the peribronchiolar extension of metaplastic bronchiolar epithelium into adjacent alveoli (asterisk). H&E

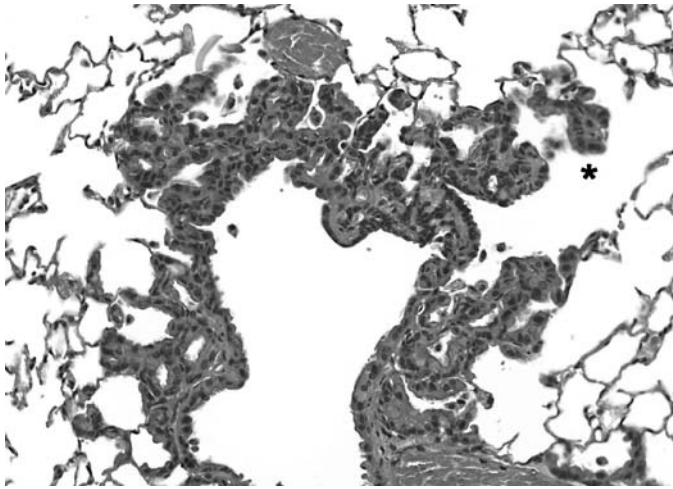


PLATE 13

Higher magnification of Plate 12. Note the metaplastic cuboidal bronchiolar cells extending into adjacent alveoli (asterisks). H&E

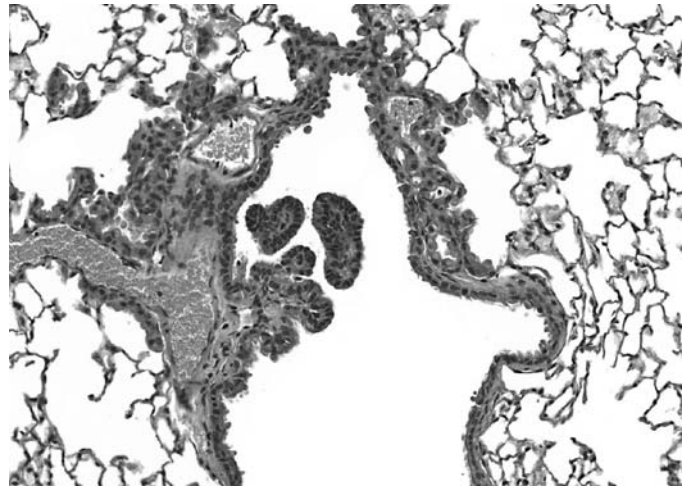


PLATE 14

Lung of a female B6C3F1 mouse exposed to 125 ppm cumene in the 2-year inhalation study. Small papillary projections were formed by the hyperplastic bronchiolar epithelium. H&E

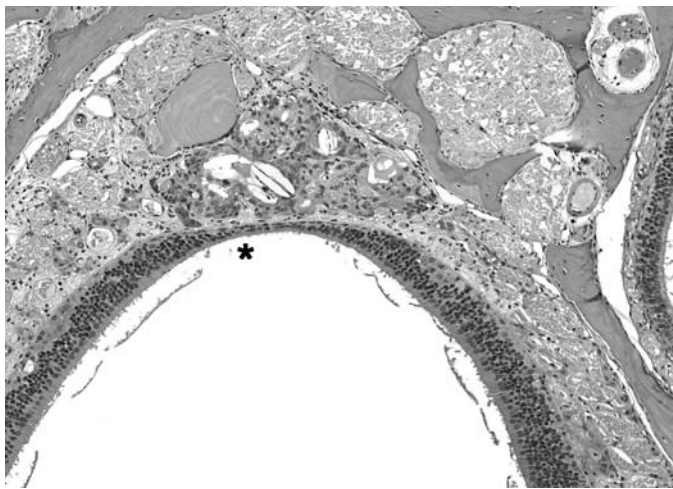


PLATE 15

Nasal tissues in Level III of a male B6C3F1 mouse exposed to 1,000 ppm cumene in the 2-year inhalation study. Localized atrophic olfactory epithelium has pronounced thinning of the mucosa (asterisk). The adjacent mucosa has basal cell hyperplasia. Submucosal Bowman's glands are hyperplastic with granulomatous inflammation, lipoid cysts, and clefts. H&E

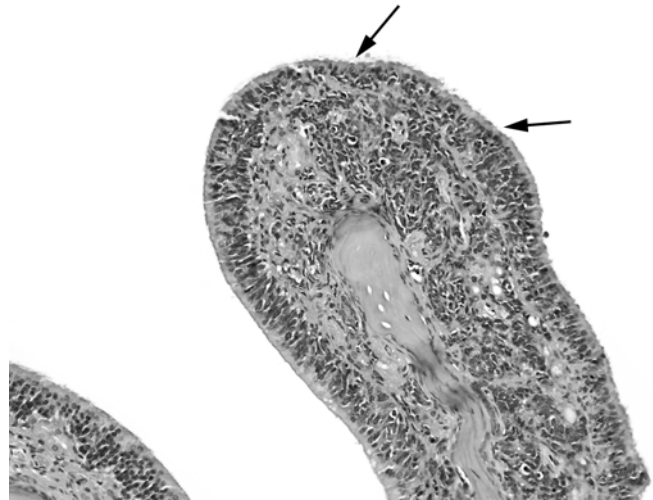


PLATE 16

Olfactory epithelium with atypical hyperplasia in nasal Level III of a male B6C3F1 mouse exposed to 1,000 ppm cumene in the 2-year inhalation study. Note the increased numbers of basophilic cells along the basement membrane resulting in disorganization of the olfactory epithelium and extension into the adjacent submucosal tissues (arrows). H&E

DISCUSSION AND CONCLUSIONS

Cumene was nominated for study by the National Institute of Environmental Health Sciences because of its high production volume, presence in gasoline and other fuels, potential for human exposure, and lack of existing carcinogenicity test data. Cumene is a central nervous system depressant (Andrews and Snyder, 1993). At high exposure concentrations in the 2-week, 3-month, and 2-year rat and mouse studies, cumene induced ataxia, but surviving animals recovered and developed tolerance after a few days. Microscopic alterations in the peripheral and central nervous systems were not observed. Cushman *et al.* (1995) reported changes in the functional observational battery test in male and female rats 1 hour after a single 6-hour exposure to 1,200 ppm cumene; the changes included gait abnormalities, increased horizontal activity, decreased mean rectal temperature, and decreased toe-pinch withdrawal reflexes. The alterations in behavioral or motor activity disappeared within 24 hours. The findings were typical of those commonly observed following exposure to alkylbenzenes, such as toluene and ethylbenzene (Andrews and Snyder, 1993; Tegeris and Balster, 1994).

The highest exposure concentration selected for male and female rats and male mice in the 2-year studies was 1,000 ppm. Deaths, presumably from central nervous system depression, occurred at higher exposure concentrations in the 2-week studies, and deaths occurred at 1,000 ppm in the 3-month female mouse study, resulting in the selection of 500 ppm as the highest exposure concentration for the 2-year female mouse study.

In the 3-month rat study, the severities of renal cortical (proximal) tubular cell hyaline droplet accumulation and regeneration (hypertrophy, hyperplasia) in males increased with increasing exposure concentration. The incidences of medullary granular casts in the 250, 500, and 1,000 ppm male rats were significantly increased, and the severity of this lesion was generally exposure-concentration-related. Levels of α 2u-globulin in the kidney also were significantly greater in male rats exposed to 125 ppm or greater compared to the chamber controls. The absolute and relative kidney weights of exposed

groups of male rats were increased. Relative kidney weights of female rats exposed to 250 ppm or greater were significantly increased, but no accompanying histopathology changes were observed. These changes were consistent with α 2u-globulin nephropathy.

In the 2-year study, cumene induced significantly increased incidences of olfactory epithelium basal cell hyperplasia in the nose for all exposed groups of rats and hyperplasia of the respiratory epithelium in all exposed groups of male rats and in 1,000 ppm females. Respiratory epithelium adenomas were observed in all exposed groups. A positive trend in the incidences of respiratory epithelium adenoma occurred in male rats but not in female rats. The incidences of adenoma in the 1,000 ppm males and females were lower than those seen at 500 ppm. However, if the incidences of respiratory epithelium hyperplasia and adenoma are considered together, exposure concentration-related increase trends are apparent. Hyperplasia of the respiratory epithelium and adenoma form a morphologic continuum. The areas of respiratory epithelial hyperplasia and adenomas were discrete foci with few or no abnormalities in the adjacent respiratory epithelium. Male rats appeared to be more sensitive than females to the progression from hyperplasia to adenoma caused by cumene. Progression of nasal respiratory epithelial adenomas to malignancy has been described in the literature (Brown *et al.*, 1991; Morgan and Harkema, 1996). No evidence of malignant progression was observed in the present studies. Hyperplasia of the respiratory epithelium in the nose was not observed in rats in the 3-month study nor in the 3-month or 2-year mouse studies.

In the 2-year rat study, incidences of renal tubule adenoma or carcinoma (combined) were increased in exposed males, and the increase was significant at 500 ppm. The renal tubular neoplasms were considered related to cumene exposure because spontaneous incidences of renal neoplasms in untreated male F344/N rats in inhalation studies are very low. Along with renal neoplasms, the incidences of renal papilla mineralization and renal pelvis transitional epithelium hyperplasia

were also increased in exposed male rats. Renal tubule hyperplasia was considered a preneoplastic lesion. The pathogenesis underlying the renal lesions in male rats in the 2-year study is likely similar to that observed with many structurally related chemicals, such as ethylbenzene. Cumene or a metabolite is thought to bind to α_2 -globulin causing the protein to resist lysosomal degradation. The pathogenesis process probably involves accumulation of protein droplets in the S2 segment of the proximal tubules, necrosis secondary to lysosomal overload, and increased cell proliferation. The continuing necrosis eventually overwhelms the capacity of the kidney to remove necrotic debris in tubular lumens, resulting in granular casts and mineralization of the papillary tubules. The histopathologic changes ultimately lead to the development of renal neoplasm. The pathogenesis of this syndrome has been widely discussed in the literature (Swenberg *et al.*, 1989; Montgomery and Seely, 1990; Melnick, 1992; Hard *et al.*, 1993; Goldstein and Schnellmann, 1996; Lehman-Mckeeman, 1997; Kohn and Melnick, 1999; Schnellmann, 2001). These changes were not seen in female rats, male or female mice, or humans.

In the 2-year mouse study, chemical-related neoplasms and nonneoplastic lesions in the lung included increased incidences in all exposed groups of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, alveolar/bronchiolar epithelium metaplasia, and bronchiole hyperplasia. The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) in male and female mice exceeded the historical control range at all exposure concentrations, indicating an unequivocal carcinogenic effect. Alveolar/bronchiolar epithelial hyperplasia is considered a preneoplastic change and may progress to adenoma and then carcinoma (Foley *et al.*, 1991; Dixon *et al.*, 1999).

In the 2-year mouse study, cumene exposure induced olfactory epithelium lesions in the nose, including atrophy, basal cell hyperplasia, atypical hyperplasia, and gland hyperplasia, in males and females. Olfactory atypical basal cell hyperplasia may have represented a preneoplastic change. However, no nasal olfactory epithelium neoplasms were found in this study. A significant increase in the incidence of squamous metaplasia of the respiratory epithelium occurred in 500 ppm females.

In the 2-year mouse study, cumene exposure affected the livers of females to a greater extent than males.

Exposure concentration-related increases in the incidences of hepatocellular adenoma or carcinoma (combined) were observed in female mice, and the increase in the 500 ppm group was significant. In males exposed to 500 or 1,000 ppm, the incidences of eosinophilic focus were increased. Ethylbenzene, which is structurally similar to cumene, also caused an increased incidence of hepatocellular neoplasms only in female mice (Chan *et al.*, 1998; NTP, 1999).

In the 2-year mouse study, increased incidences of forestomach epithelial hyperplasia and inflammation were observed in exposed males. The increases were probably related to irritating effects of cumene, and similar lesions are commonly seen in NTP inhalation studies, presumably resulting from ingestion of the chemical from fur or clearance from the lung through microciliary action.

Cumene is an alkylbenzene structurally similar to benzene, toluene, styrene, xylene, and ethylbenzene (Andrews and Snyder, 1993). Like other alkylbenzenes, cumene may be metabolized by oxidation of the side chain or by ring oxidation. Both biotransformations are catalyzed by cytochromes P450. However, a pathway for metabolic activation of cumene to an intermediate capable of reacting with protein or DNA is not obvious. There are no structural alerts. Cumene is a relatively small aromatic molecule, probably a substrate for CYP2E1 and CYP2F2. CYP2F2 has been located in Clara cells in mouse lung using immunohistochemical techniques. There was little immunological reaction for CYP2F in Clara cells from rat or hamster lungs. This observation correlates with rates of naphthalene biotransformation in the three species (Buckpitt *et al.*, 1995). The biotransformations of styrene have been extensively studied. While epoxidation of the vinyl group is the major biotransformation pathway, a ring-hydroxylated product, 4-vinylphenol, is also formed. Vinylphenol is more toxic than styrene oxide but requires further biotransformation to be toxic (Cruzan *et al.*, 2002). The phenol is more toxic to mouse lung than rat lung. The toxicity can be prevented by treatment with a CYP2F inhibitor, 5-phenyl-1-pentyne (Cruzan *et al.*, 2002). The observation that mice exhale more $^{14}\text{CO}_2$ than rats when dosed with ring-labeled [^{14}C]-styrene implies that the aromatic ring is broken and is consistent with more extensive metabolism in mice (Boogaard *et al.*, 2000). With this as a background, a pathway for activation of cumene can be proposed (Figure 6). Ring-hydroxylation to isopropylphenol is the proposed first step. There are at least three possible activated intermediates from

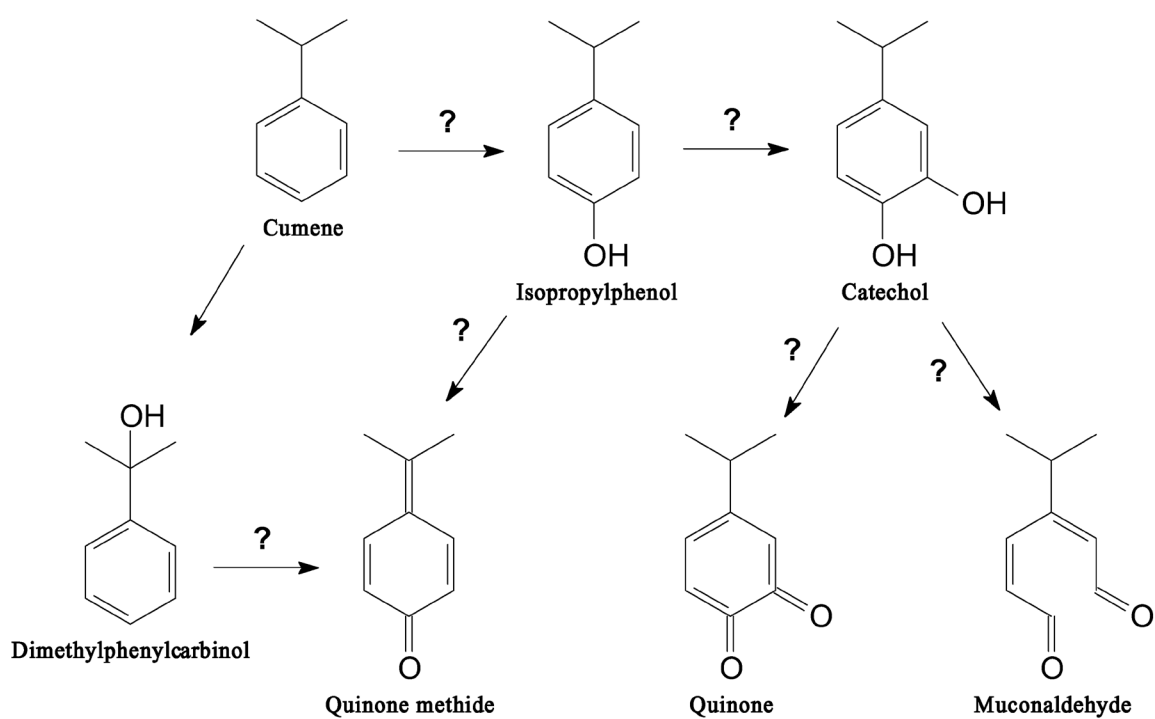


FIGURE 6
Possible Reactive Metabolites of Cumene

further biotransformation of the phenol: oxidation to a quinone methide, ring-opening to a muconaldehyde, or hydroxylation to a catechol and oxidation to a quinone. Biotransformation of cumene by CYP2F2 in mouse lung and nasal tissue and CYP2F4 in rat nasal tissue could lead to the observed species difference.

Studies of the absorption, distribution, metabolism, and excretion of cumene are ongoing and may provide more insight on the biotransformation and bioactivation. Preliminary results have confirmed the major urinary metabolites as described in Figure 6. Exhaled organic volatiles are composed of an approximately 1:1 mixture of cumene and α -methylstyrene. α -Methylstyrene has not previously been reported as a metabolite of cumene; however, the urinary metabolites 2-phenyl-1,2-propanediol and 2-hydroxyphenylpropanoic acid are likely to be derived from the epoxide of α -methylstyrene. Whether α -methylstyrene requires ring-hydroxylation as an activating step in producing reactive intermediates as described for styrene is not known; however, certain aspects of the toxicity/carcinogenicity of inhaled α -methylstyrene (NTP, 2007) are similar to those of cumene. For example, in rats, cumene at 1,000 ppm induced olfactory epithelial hyperplasia and respiratory epithelial neoplasms in males and females and α 2u-globulin nephropathy and renal tubular neoplasms in males. α -Methylstyrene at 1,000 ppm also induced olfactory epithelial hyperplasia in males and females, although it did not appear to affect the respiratory epithelium, as did cumene, and caused α 2u-globulin nephropathy and renal tubular neoplasms in males. In mice, cumene at 500 and 1,000 ppm induced olfactory epithelial hyperplasia and other nasal lesions in males and females, hepatocellular neoplasms in females, and hepatic eosinophilic foci in males. α -Methylstyrene at 600 ppm also induced olfactory epithelial hyperplasia in males and females and hepatocellular neoplasms in females. In contrast, the marked carcinogenic effects in the lung of male and female mice caused by cumene did not occur with α -methylstyrene. Thus, even though cumene can be metabolized to α -methylstyrene, the toxic and carcinogenic effects of cumene are more widespread than those of α -methylstyrene. Therefore, α -methylstyrene can not be the sole active contributor to the carcinogenicity of cumene. It is not known how α -methylstyrene interacts with cellular macromolecules in its carcinogenesis process, although a metabolic activation via styrene and styrene oxide has been suggested. There is no evidence that α -methylstyrene binds

to DNA or protein. α -Methylstyrene is not mutagenic in *Salmonella* tests with or without S9. α -Methylstyrene did not induce micronuclei in erythrocytes in male mice, but in female mice, a significant increase in micronucleated normochromatic erythrocytes was seen at the highest concentration tested (NTP, 2007).

The present studies demonstrated that cumene is a multisite carcinogen both in mice, inducing neoplasms in the lung and liver, and in rats, inducing neoplasms in the nose and kidney. Multisite, multispecies carcinogens are frequently genotoxic carcinogens, but standard assays for genotoxicity did not detect cumene as a genotoxicant. Cumene was not mutagenic in the Ames test, and it did not induce micronuclei, indicative of chromosomal damage, in erythrocytes of mice exposed to cumene for 3 months by inhalation (Appendix E). Although positive results in the Ames assay and the 3-month mouse erythrocyte micronucleus assay are highly predictive of carcinogenicity, negative results are not predictive of noncarcinogenicity (Zeiger *et al.*, 1990; Witt *et al.*, 2000). Because of the extensive metabolism that cumene undergoes *in vivo*, it is possible that the proximate carcinogen is not generated in the *in vitro* Ames test, even with the addition of exogenous metabolic activation enzymes. Cumene did, however, induce a small, but statistically significant, increase in micronucleated erythrocytes in bone marrow of male rats treated by intraperitoneal injection. The contrast with the results in the 3-month micronucleus test in mice might be explained by differences in route and dosage between the two tests, such that greater exposure of the proerythrocyte target cells in the bone marrow was achieved in the rat study. The significantly increased frequencies of *K-ras* and *p53* mutations seen in lung neoplasms of mice exposed to cumene in this study (Appendix L) clearly imply genetic events related to cumene exposure, because these mutations were not observed in spontaneous neoplasms in the chamber control mice. Similar findings of mutations in cancer genes and negative results in *Salmonella* and mouse micronucleus tests have been observed in previous studies. For example, with the nongenotoxic agent oxazepam, mutations in the *B-catenin* gene were related to cytochrome p450 induction and oxidative stress. Also, chloroprene, which is inactive in *Salmonella* and mouse micronucleus studies, induced lung tumors with signature *K-ras* A to T transversions caused by adenine adducts related to a specific chloroprene metabolite.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma (combined). Increased incidences of interstitial cell adenoma of the testis may have been related to exposure to cumene. There was *some evidence of carcinogenic activity* of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose. There was *clear evidence of carcinogenic activity* of cumene in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. The increased incidences of hemangiosarcoma in the spleen and follicular

cell adenoma in the thyroid gland in male mice may have been related to cumene exposure. There was *clear evidence of carcinogenic activity* of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Increased incidences of hepatocellular adenoma or carcinoma (combined) in female mice were also considered to be related to exposure to cumene.

Exposure of male rats to cumene resulted in nonneoplastic lesions of the kidney characteristic of α 2u-globulin accumulation. Exposure to cumene resulted in nonneoplastic lesions in the nose of male and female rats; the lung, nose, liver, and forestomach of male mice; and the lung and nose of female mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Report Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF CUMENE

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	20	24	21	24
Natural deaths	4	3	2	2
Survivors				
Terminal sacrifice	26	23	27	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(49)	(49)	(49)
Intestine large, colon	(49)	(50)	(50)	(50)
Intestine large, rectum	(49)	(50)	(50)	(50)
Intestine small, duodenum	(49)	(49)	(49)	(49)
Intestine small, ileum	(49)	(47)	(48)	(49)
Intestine small, jejunum	(48)	(47)	(48)	(49)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate				1 (2%)
Hepatocellular adenoma			1 (2%)	
Hepatocellular carcinoma				2 (4%)
Mesentery	(7)	(13)	(10)	(6)
Carcinoma, metastatic, prostate				1 (17%)
Pancreas	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(3)	(1)	(3)
Papilloma		1 (33%)		
Tooth		(1)		
Cardiovascular System				
Blood vessel	(1)	(1)	(1)	
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	1 (2%)
Carcinoma, metastatic, prostate				1 (2%)
Pericardium, carcinoma, metastatic, lung	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		2 (4%)	3 (6%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	7 (14%)	6 (12%)	9 (18%)	8 (16%)
Pheochromocytoma malignant	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Bilateral, pheochromocytoma benign		1 (2%)		3 (6%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Carcinoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Parathyroid gland	(45)	(49)	(49)	(48)
Pituitary gland	(50)	(50)	(49)	(50)
Adenoma	36 (72%)	38 (76%)	25 (51%)	30 (60%)

TABLE A1

Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	3 (6%)	7 (14%)	1 (2%)	4 (8%)
C-cell, carcinoma	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Follicular cell, adenoma		1 (2%)		1 (2%)
Follicular cell, carcinoma	2 (4%)			2 (4%)
General Body System				
Peritoneum	(2)	(2)	(4)	(2)
Carcinoma, metastatic, prostate				1 (50%)
Carcinoma, metastatic, urinary bladder			1 (25%)	
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate				1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Carcinoma		3 (6%)	1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Carcinoma, metastatic, urinary bladder			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate				1 (2%)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (36%)	24 (48%)	27 (54%)	37 (74%)
Interstitial cell, adenoma	18 (36%)	14 (28%)	13 (26%)	9 (18%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(10)	(8)	(14)
Deep cervical, carcinoma, metastatic, skin		1 (10%)		
Lymph node, bronchial	(7)	(12)	(11)	(8)
Lymph node, mandibular	(1)	(3)		(1)
Lymph node, mediastinal	(34)	(32)	(34)	(34)
Carcinoma, metastatic, thyroid gland	1 (3%)	1 (3%)		
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate				1 (2%)
Thymus	(49)	(49)	(50)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)		2 (4%)	
Carcinoma, multiple	1 (2%)			
Fibroadenoma		1 (2%)	3 (6%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Basal cell carcinoma			2 (4%)	
Keratoacanthoma	4 (8%)	1 (2%)	2 (4%)	2 (4%)
Keratoacanthoma, multiple		1 (2%)		1 (2%)
Squamous cell papilloma			1 (2%)	
Trichoepithelioma				1 (2%)
Sebaceous gland, adenoma	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Sebacous gland, carcinoma		1 (2%)		
Subcutaneous tissue, carcinoma, metastatic, Harderian gland		1 (2%)		
Subcutaneous tissue, fibroma	3 (6%)	3 (6%)	3 (6%)	4 (8%)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, lipoma		1 (2%)	1 (2%)	
Subcutaneous tissue, schwannoma, benign				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, carcinoma, metastatic, Zymbal's gland	1 (2%)			
Skeletal muscle	(1)	(2)	(2)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)	
Carcinoma, metastatic, lung	1 (100%)			
Carcinoma, metastatic, prostate				1 (100%)
Carcinoma, metastatic, urinary bladder			1 (50%)	
Rhabdomyosarcoma		1 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor malignant		1 (2%)		
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma				2 (4%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)		1 (2%)	1 (2%)
Carcinoma, metastatic, prostate				1 (2%)
Carcinoma, metastatic, skin		1 (2%)	1 (2%)	
Carcinoma, metastatic, thyroid gland				1 (2%)
Carcinoma, metastatic, urinary bladder			1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)			1 (2%)
Nose	(50)	(50)	(49)	(50)
Glands, respiratory epithelium, adenoma			1 (2%)	
Respiratory epithelium, adenoma		6 (12%)	15 (31%)	4 (8%)
Respiratory epithelium, adenoma, multiple		1 (2%)	2 (4%)	6 (12%)
Pleura	(5)	(3)	(5)	(6)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (20%)	
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(49)	(50)
Carcinoma, metastatic, Harderian gland		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Zymbal's gland	(1)			(2)
Carcinoma	1 (100%)			2 (100%)

TABLE A1

Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate				1 (2%)
Lipoma	1 (2%)			1 (2%)
Bilateral, renal tubule, carcinoma			1 (2%)	
Renal tubule, adenoma	1 (2%)	4 (8%)	5 (10%)	4 (8%)
Renal tubule, carcinoma	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Ureter		(1)		(1)
Carcinoma, metastatic, prostate				1 (100%)
Urethra			(1)	(1)
Carcinoma, metastatic, prostate				1 (100%)
Urinary bladder	(50)	(50)	(49)	(50)
Transitional epithelium, carcinoma			2 (4%)	1 (2%)
Transitional epithelium, papilloma				2 (4%)
Systemic Lesions				
Multiple organs ^b	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	26 (52%)	32 (64%)	31 (62%)	28 (56%)
Lymphoma malignant				1 (2%)
Mesothelioma malignant	3 (6%)	2 (4%)	3 (6%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	50	50	50	50
Total primary neoplasms	135	163	166	176
Total animals with benign neoplasms	48	48	47	50
Total benign neoplasms	95	117	117	125
Total animals with malignant neoplasms	36	37	38	39
Total malignant neoplasms	40	46	49	51
Total animals with metastatic neoplasms	4	4	3	4
Total metastatic neoplasms	5	6	8	16

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Adrenal Cortex: Adenoma				
Overall rate ^a	0/50 (0%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate ^b	0.0%	5.1%	7.3%	0.0%
Terminal rate ^c	0/26 (0%)	1/23 (4%)	2/27 (7%)	0/24 (0%)
First incidence (days)	— ^e	665	626	— ^f
Poly-3 test ^d	P=0.519N	P=0.226	P=0.117	—
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	7/50 (14%)	7/50 (14%)	9/50 (18%)	11/50 (22%)
Adjusted rate	16.7%	17.4%	21.5%	25.9%
Terminal rate	5/26 (19%)	4/23 (17%)	6/27 (22%)	8/24 (33%)
First incidence (days)	647	624	600	688
Poly-3 test	P=0.149	P=0.583	P=0.387	P=0.221
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	7/50 (14%)	7/50 (14%)	10/50 (20%)	13/50 (26%)
Adjusted rate	16.7%	17.4%	23.8%	30.5%
Terminal rate	5/26 (19%)	4/23 (17%)	6/27 (22%)	9/24 (38%)
First incidence (days)	647	624	600	688
Poly-3 test	P=0.054	P=0.583	P=0.293	P=0.103
Kidney (Renal Tubule): Adenoma				
Overall rate	1/50 (2%)	4/50 (8%)	5/50 (10%)	4/50 (8%)
Adjusted rate	2.4%	10.0%	12.1%	9.3%
Terminal rate	1/26 (4%)	1/23 (4%)	2/27 (7%)	2/24 (8%)
First incidence (days)	729 (T)	665	679	635
Poly-3 test	P=0.219	P=0.165	P=0.099	P=0.187
Kidney (Renal Tubule): Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.4%	2.5%	7.3%	7.0%
Terminal rate	1/26 (4%)	1/23 (4%)	2/27 (7%)	2/24 (8%)
First incidence (days)	729 (T)	729 (T)	639	618
Poly-3 test	P=0.180	P=0.749	P=0.302	P=0.314
Kidney (Renal Tubule): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	8/50 (16%)	7/50 (14%)
Adjusted rate	4.8%	12.5%	19.2%	16.2%
Terminal rate	2/26 (8%)	2/23 (9%)	4/27 (15%)	4/24 (17%)
First incidence (days)	729 (T)	665	639	618
Poly-3 test	P=0.087	P=0.198	P=0.044	P=0.087
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.4%	0.0%	2.4%	7.0%
Terminal rate	1/26 (4%)	0/23 (0%)	1/27 (4%)	1/24 (4%)
First incidence (days)	729 (T)	—	729 (T)	674
Poly-3 test	P=0.104	P=0.511N	P=0.758	P=0.314
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	5/50 (10%)
Adjusted rate	4.8%	5.1%	7.3%	11.7%
Terminal rate	2/26 (8%)	2/23 (9%)	2/27 (7%)	3/24 (13%)
First incidence (days)	729 (T)	729 (T)	670	674
Poly-3 test	P=0.125	P=0.675	P=0.496	P=0.226

TABLE A2

Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Mammary Gland: Fibroadenoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	2.5%	7.3%	4.7%
Terminal rate	0/26 (0%)	1/23 (4%)	3/27 (11%)	2/24 (8%)
First incidence (days)	—	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.174	P=0.489	P=0.115	P=0.241
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	5/50 (10%)	2/50 (4%)
Adjusted rate	4.8%	2.5%	12.2%	4.7%
Terminal rate	1/26 (4%)	1/23 (4%)	5/27 (19%)	2/24 (8%)
First incidence (days)	628	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.479	P=0.522N	P=0.205	P=0.691N
Nose: Adenoma				
Overall rate	0/50 (0%)	7/50 (14%)	18/49 (37%)	10/50 (20%)
Adjusted rate	0.0%	17.6%	43.2%	23.3%
Terminal rate	0/26 (0%)	5/23 (22%)	13/27 (48%)	7/24 (29%)
First incidence (days)	—	639	638	674
Poly-3 test	P=0.004	P=0.006	P<0.001	P<0.001
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	3/50 (6%)
Adjusted rate	7.2%	7.6%	9.7%	7.1%
Terminal rate	2/26 (8%)	3/23 (13%)	3/27 (11%)	3/24 (13%)
First incidence (days)	689	729 (T)	679	729 (T)
Poly-3 test	P=0.578	P=0.636	P=0.492	P=0.657N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	36/50 (72%)	38/50 (76%)	25/49 (51%)	30/50 (60%)
Adjusted rate	76.2%	80.4%	58.4%	62.9%
Terminal rate	20/26 (77%)	18/23 (78%)	17/27 (63%)	13/24 (54%)
First incidence (days)	296	480	625	453
Poly-3 test	P=0.030N	P=0.396	P=0.048N	P=0.110N
Preputial Gland: Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	7.5%	2.4%	0.0%
Terminal rate	0/26 (0%)	1/23 (4%)	1/27 (4%)	0/24 (0%)
First incidence (days)	—	639	729 (T)	—
Poly-3 test	P=0.363N	P=0.111	P=0.497	—
Preputial Gland: Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	7.5%	2.4%	0.0%
Terminal rate	0/26 (0%)	1/23 (4%)	1/27 (4%)	0/24 (0%)
First incidence (days)	—	639	729 (T)	—
Poly-3 test	P=0.363N	P=0.111	P=0.497	—
Skin: Keratoacanthoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	9.5%	5.1%	4.9%	7.1%
Terminal rate	1/26 (4%)	2/23 (9%)	2/27 (7%)	1/24 (4%)
First incidence (days)	613	729 (T)	729 (T)	677
Poly-3 test	P=0.464N	P=0.369N	P=0.350N	P=0.496N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted rate	9.5%	5.1%	7.3%	7.1%
Terminal rate	1/26 (4%)	2/23 (9%)	3/27 (11%)	1/24 (4%)
First incidence (days)	613	729 (T)	729 (T)	677
Poly-3 test	P=0.482N	P=0.369N	P=0.516N	P=0.496N
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.4%	2.5%	7.2%	4.7%
Terminal rate	1/26 (4%)	1/23 (4%)	1/27 (4%)	2/24 (8%)
First incidence (days)	729 (T)	729 (T)	554	729 (T)
Poly-3 test	P=0.342	P=0.749	P=0.305	P=0.506
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	6/50 (12%)	5/50 (10%)
Adjusted rate	11.8%	7.6%	14.4%	11.8%
Terminal rate	2/26 (8%)	3/23 (13%)	4/27 (15%)	3/24 (13%)
First incidence (days)	613	729 (T)	554	677
Poly-3 test	P=0.478	P=0.394N	P=0.491	P=0.626N
Skin: Fibroma				
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	4/50 (8%)
Adjusted rate	7.2%	7.6%	7.3%	9.4%
Terminal rate	2/26 (8%)	2/23 (9%)	2/27 (7%)	2/24 (8%)
First incidence (days)	632	711	688	677
Poly-3 test	P=0.423	P=0.635	P=0.654	P=0.508
Skin: Fibroma or Fibrosarcoma				
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	5/50 (10%)
Adjusted rate	7.2%	7.6%	7.3%	11.7%
Terminal rate	2/26 (8%)	2/23 (9%)	2/27 (7%)	3/24 (13%)
First incidence (days)	632	711	688	677
Poly-3 test	P=0.271	P=0.635	P=0.654	P=0.364
Testes: Adenoma				
Overall rate	36/50 (72%)	38/50 (76%)	40/50 (80%)	46/50 (92%)
Adjusted rate	80.0%	84.6%	85.7%	96.1%
Terminal rate	24/26 (92%)	22/23 (96%)	25/27 (93%)	24/24 (100%)
First incidence (days)	558	536	460	541
Poly-3 test	P=0.006	P=0.370	P=0.311	P=0.007
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	7/50 (14%)	1/50 (2%)	4/50 (8%)
Adjusted rate	7.1%	17.4%	2.4%	9.4%
Terminal rate	1/26 (4%)	3/23 (13%)	0/27 (0%)	3/24 (13%)
First incidence (days)	571	589	702	677
Poly-3 test	P=0.485N	P=0.133	P=0.318N	P=0.500
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	9/50 (18%)	2/50 (4%)	6/50 (12%)
Adjusted rate	7.1%	22.0%	4.9%	14.1%
Terminal rate	1/26 (4%)	4/23 (17%)	1/27 (4%)	5/24 (21%)
First incidence (days)	571	541	702	677
Poly-3 test	P=0.432	P=0.048	P=0.516N	P=0.240

TABLE A2

Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	4.8%	2.5%	0.0%	7.0%
Terminal rate	2/26 (8%)	0/23 (0%)	0/27 (0%)	1/24 (4%)
First incidence (days)	729 (T)	686	—	677
Poly-3 test	P=0.348	P=0.518N	P=0.240N	P=0.511
Urinary Bladder: Papilloma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	2/49 (4%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	4.8%	7.1%
Terminal rate	0/26 (0%)	0/23 (0%)	0/27 (0%)	2/24 (8%)
First incidence (days)	—	—	213	677
Poly-3 test	P=0.030	—	P=0.238	P=0.122
All Organs: Mononuclear Leukemia				
Overall rate	26/50 (52%)	32/50 (64%)	31/50 (62%)	28/50 (56%)
Adjusted rate	57.8%	69.7%	65.5%	60.0%
Terminal rate	12/26 (46%)	14/23 (61%)	13/27 (48%)	11/24 (46%)
First incidence (days)	600	519	460	515
Poly-3 test	P=0.490N	P=0.160	P=0.289	P=0.501
All Organs: Malignant Mesothelioma				
Overall rate	3/50 (6%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	7.1%	5.0%	7.2%	2.4%
Terminal rate	2/26 (8%)	0/23 (0%)	1/27 (4%)	1/24 (4%)
First incidence (days)	599	575	634	729 (T)
Poly-3 test	P=0.253N	P=0.522N	P=0.659	P=0.302N
All Organs: Benign Neoplasms				
Overall rate	48/50 (96%)	48/50 (96%)	47/50 (94%)	50/50 (100%)
Adjusted rate	97.5%	98.8%	97.5%	100%
Terminal rate	26/26 (100%)	23/23 (100%)	27/27 (100%)	24/24 (100%)
First incidence (days)	296	480	460	453
Poly-3 test	P=0.261	P=0.633	P=0.770	P=0.400
All Organs: Malignant Neoplasms				
Overall rate	36/50 (72%)	37/50 (74%)	38/50 (76%)	39/50 (78%)
Adjusted rate	77.5%	78.1%	77.3%	80.4%
Terminal rate	19/26 (73%)	16/23 (70%)	17/27 (63%)	17/24 (71%)
First incidence (days)	495	445	213	515
Poly-3 test	P=0.410	P=0.576	P=0.588N	P=0.462

TABLE A2

Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100%	100%	100%	100%
Terminal rate	26/26 (100%)	23/23 (100%)	27/27 (100%)	24/24 (100%)
First incidence (days)	296	445	213	453
Poly-3 test	—	—	—	—

(T)Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, lung, nose, pancreatic islets, pituitary gland, preputial gland, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

^b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by N.

^e Not applicable; no neoplasms in animal group

^f Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Adenoma of the Nose in Control Male F344/N Rats^a

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	0/50
Decalin	0/49
Divinylbenzene	0/50
Methyl isobutyl ketone	0/50
α -Methylstyrene	0/50
Propargyl alcohol	0/49
Propylene glycol mono- <i>t</i> -butyl ether	0/50
Stoddard solvent IIC	1/50
Vanadium pentoxide	0/49
Overall Historical Incidence: Inhalation Studies	
Total (%)	1/447 (0.2%)
Mean \pm standard deviation	0.2% \pm 0.7%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	2/1,439 (0.1%)
Mean \pm standard deviation	0.1% \pm 0.5%
Range	0%-2%

^a Data as of March 2, 2007

TABLE A3b
Historical Incidence of Kidney Neoplasms in Control Male F344/N Rats^a

Study	Incidence in Controls			
	Lipoma	Renal Tubule Adenoma	Renal Tubule Carcinoma	Renal Tubule Adenoma or Carcinoma
Historical Incidence: Inhalation Studies				
Cumene	1/50	1/50	1/50	2/50
Decalin	0/50	1/50	0/50	1/50
Divinylbenzene	0/50	0/50	0/50	0/50
Methyl isobutyl ketone	0/50	0/50	0/50	0/50
α -Methylstyrene	0/50	0/50	0/50	0/50
Propargyl alcohol	0/49	0/49	0/49	0/49
Propylene glycol mono- <i>t</i> -butyl ether	0/50	1/50	0/50	1/50
Stoddard solvent IIC	0/50	0/50	1/50	1/50
Vanadium pentoxide	0/50	1/50	0/50	1/50
Overall Historical Incidence: Inhalation Studies				
Total (%)	1/449 (0.2%)	4/449 (0.9%)	2/449 (0.5%)	6/449 (1.3%)
Mean \pm standard deviation	0.2% \pm 0.7%	0.9% \pm 1.0%	0.4% \pm 0.9%	1.3% \pm 1.4%
Range	0%-2%	0%-2%	0%-2%	0%-4%
Overall Historical Incidence: All Routes				
Total (%)	2/1,436 (0.1%)	8/1,436 (0.6%)	2/1,436 (0.1%)	10/1,436 (0.7%)
Mean \pm standard deviation	0.1% \pm 0.5%	0.6% \pm 0.8%	0.1% \pm 0.5%	0.7% \pm 1.0%
Range	0%-2%	0%-2%	0%-2%	0%-4%

^a Data as of March 2, 2007

TABLE A3c

Historical Incidence of Adenoma of the Testis in Control Male F344/N Rats^a

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	36/50
Decalin	40/50
Divinylbenzene	38/50
Methyl isobutyl ketone	42/50
α -Methylstyrene	33/50
Propargyl alcohol	38/49
Propylene glycol mono- <i>t</i> -butyl ether	41/50
Stoddard solvent IIC	41/50
Vanadium pentoxide	36/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	345/449 (76.8%)
Mean \pm standard deviation	76.8% \pm 5.9%
Range	66%-84%
Overall Historical Incidence: All Routes	
Total (%)	1,242/1,449 (85.7%)
Mean \pm standard deviation	85.5% \pm 8.7%
Range	66%-98%

^a Data as of March 5, 2007

TABLE A4

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	20	24	21	24
Natural deaths	4	3	2	2
Survivors				
Terminal sacrifice	26	23	27	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Foreign body		1 (2%)		
Intestine large, cecum	(49)	(49)	(49)	(49)
Intestine large, colon	(49)	(50)	(50)	(50)
Diverticulum	1 (2%)			
Intestine large, rectum	(49)	(50)	(50)	(50)
Intestine small, duodenum	(49)	(49)	(49)	(49)
Intestine small, ileum	(49)	(47)	(48)	(49)
Intestine small, jejunum	(48)	(47)	(48)	(49)
Inflammation, chronic active	1 (2%)			
Thrombosis	1 (2%)			
Ulcer	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Basophilic focus	7 (14%)	4 (8%)	8 (16%)	6 (12%)
Clear cell focus	12 (24%)	6 (12%)	11 (22%)	11 (22%)
Degeneration, cystic	3 (6%)	1 (2%)	4 (8%)	6 (12%)
Eosinophilic focus		1 (2%)	3 (6%)	
Eosinophilic focus, multiple			1 (2%)	
Fatty change, diffuse		1 (2%)		
Hepatodiaphragmatic nodule	3 (6%)	8 (16%)	2 (4%)	3 (6%)
Inflammation, suppurative		1 (2%)		
Mixed cell focus	1 (2%)		3 (6%)	1 (2%)
Necrosis	3 (6%)	3 (6%)	3 (6%)	3 (6%)
Vacuolization cytoplasmic	6 (12%)	2 (4%)	3 (6%)	2 (4%)
Bile duct, hyperplasia	7 (14%)	4 (8%)	12 (24%)	7 (14%)
Hepatocyte, regeneration	1 (2%)			
Oval cell, hyperplasia				1 (2%)
Periportal, inflammation, chronic			2 (4%)	
Mesentery	(7)	(13)	(10)	(6)
Hemorrhage		1 (8%)		1 (17%)
Necrosis	6 (86%)	10 (77%)	10 (100%)	3 (50%)
Fat, hemorrhage	1 (14%)			1 (17%)
Fat, necrosis		1 (8%)		
Pancreas	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Vacuolization cytoplasmic		1 (2%)		
Acinus, atrophy	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Acinus, hyperplasia			1 (2%)	3 (6%)

^a Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Inflammation, suppurative			2 (4%)	
Necrosis	1 (2%)			
Ulcer	3 (6%)	7 (14%)	1 (2%)	8 (16%)
Epithelium, hyperplasia	6 (12%)	11 (22%)	7 (14%)	10 (20%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Hyperplasia, lymphoid			1 (2%)	
Mineralization	1 (2%)			
Ulcer	3 (6%)		1 (2%)	1 (2%)
Tongue	(1)	(3)	(1)	(3)
Epithelium, hyperplasia	1 (100%)	2 (67%)	1 (100%)	3 (100%)
Tooth		(1)		
Inflammation		1 (100%)		
Cardiovascular System				
Blood vessel	(1)	(1)	(1)	
Mineralization	1 (100%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	9 (18%)	7 (14%)	12 (24%)	11 (22%)
Atrium, thrombosis	3 (6%)	6 (12%)	4 (8%)	4 (8%)
Valve, cardiomyopathy	1 (2%)			
Valve, hemorrhage			1 (2%)	
Valve, thrombosis	1 (2%)			
Ventricle, hypertrophy				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	17 (34%)	16 (32%)	10 (20%)	10 (20%)
Hypertrophy			1 (2%)	
Vacuolization cytoplasmic	8 (16%)	5 (10%)	7 (14%)	4 (8%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	11 (22%)	13 (26%)	18 (36%)	12 (24%)
Bilateral, hyperplasia				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)		
Parathyroid gland	(45)	(49)	(49)	(48)
Hyperplasia	1 (2%)	1 (2%)		
Pituitary gland	(50)	(50)	(49)	(50)
Cyst		1 (2%)	2 (4%)	
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hyperplasia	6 (12%)	7 (14%)	13 (27%)	6 (12%)
Pars intermedia, hyperplasia	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	7 (14%)	11 (22%)	6 (12%)	8 (16%)
Follicular cell, cyst		1 (2%)		
Follicular cell, hyperplasia			1 (2%)	
General Body System				
Peritoneum	(2)	(2)	(4)	(2)

TABLE A4

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)		2 (4%)	
Hyperplasia				1 (2%)
Inflammation, suppurative			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	1 (2%)	2 (4%)
Inflammation, suppurative	42 (84%)	32 (64%)	27 (54%)	28 (56%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation		1 (2%)		
Hyperplasia		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Necrosis			1 (2%)	
Artery, inflammation, chronic active		1 (2%)	2 (4%)	
Bilateral, interstitial cell, hyperplasia				1 (2%)
Germinal epithelium, atrophy	6 (12%)	5 (10%)	3 (6%)	6 (12%)
Interstitial cell, hyperplasia	12 (24%)	18 (36%)	19 (38%)	9 (18%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(10)	(8)	(14)
Deep cervical, ectasia		1 (10%)		1 (7%)
Deep cervical, hemorrhage				1 (7%)
Deep cervical, hyperplasia, lymphoid			1 (13%)	2 (14%)
Pancreatic, ectasia			1 (13%)	1 (7%)
Pancreatic, hyperplasia, lymphoid			1 (13%)	
Pancreatic, inflammation, granulomatous			1 (13%)	
Lymph node, bronchial	(7)	(12)	(11)	(8)
Ectasia	1 (14%)	1 (8%)		
Hyperplasia, lymphoid	1 (14%)	1 (8%)	1 (9%)	
Lymph node, mandibular	(1)	(3)		(1)
Ectasia	1 (100%)	2 (67%)		
Metaplasia, osseous	1 (100%)			
Lymph node, mediastinal	(34)	(32)	(34)	(34)
Angiectasis				1 (3%)
Hemorrhage		1 (3%)		
Hyperplasia, lymphoid	1 (3%)		2 (6%)	
Inflammation, suppurative	1 (3%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemorrhage		2 (4%)		1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen				1 (2%)
Fibrosis	2 (4%)	2 (4%)	7 (14%)	2 (4%)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	4 (8%)	4 (8%)	2 (4%)	4 (8%)
Necrosis	1 (2%)	3 (6%)	1 (2%)	
Thymus	(49)	(49)	(50)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	2 (4%)		2 (4%)	1 (2%)
Epithelium, hyperplasia	1 (2%)		1 (2%)	1 (2%)

TABLE A4

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)		2 (4%)
Hyperkeratosis	1 (2%)		1 (2%)	
Inflammation, chronic				1 (2%)
Ulcer				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis			1 (2%)	
Skeletal muscle	(1)	(2)	(2)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	7 (14%)	16 (32%)	6 (12%)	6 (12%)
Hemorrhage	5 (10%)	7 (14%)	3 (6%)	1 (2%)
Necrosis		1 (2%)		
Thrombosis		1 (2%)		
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	6 (12%)	3 (6%)		3 (6%)
Inflammation, suppurative	4 (8%)	7 (14%)	1 (2%)	3 (6%)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Epiglottis, hyperplasia	1 (2%)			
Epiglottis, metaplasia, squamous	1 (2%)		1 (2%)	2 (4%)
Respiratory epithelium, hyperplasia	3 (6%)	2 (4%)		1 (2%)
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)			
Hemorrhage	3 (6%)	7 (14%)	3 (6%)	
Inflammation			1 (2%)	
Inflammation, suppurative	1 (2%)	1 (2%)		
Inflammation, granulomatous		1 (2%)		
Inflammation, chronic	3 (6%)	3 (6%)	6 (12%)	5 (10%)
Thrombosis		1 (2%)		
Alveolar epithelium, hyperplasia	13 (26%)	11 (22%)	10 (20%)	10 (20%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Alveolar epithelium, metaplasia, mucous				1 (2%)
Alveolus, emphysema				1 (2%)
Alveolus, foreign body		1 (2%)		
Alveolus, infiltration cellular, histiocyte	9 (18%)	7 (14%)	4 (8%)	14 (28%)
Alveolus, proteinosis				1 (2%)
Artery, mineralization	2 (4%)			
Artery, thrombosis	1 (2%)			
Bronchiole, hyperplasia		1 (2%)		
Bronchiole, inflammation, chronic		1 (2%)	1 (2%)	
Interstitial, fibrosis		2 (4%)	1 (2%)	1 (2%)
Nose	(50)	(50)	(49)	(50)
Foreign body	5 (10%)	5 (10%)	3 (6%)	4 (8%)
Hyperplasia, basal cell				1 (2%)
Inflammation, suppurative	7 (14%)	8 (16%)	8 (16%)	6 (12%)
Inflammation, chronic	5 (10%)	1 (2%)		

TABLE A4

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Respiratory System (continued)				
Nose (continued)	(50)	(50)	(49)	(50)
Epithelium, nasolacrimal duct, metaplasia, squamous		2 (4%)	1 (2%)	
Glands, olfactory epithelium, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Glands, respiratory epithelium, dilatation	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Glands, respiratory epithelium, hyperplasia			2 (4%)	4 (8%)
Goblet cell, olfactory epithelium, hyperplasia			1 (2%)	
Goblet cell, hyperplasia	3 (6%)	11 (22%)	7 (14%)	5 (10%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	2 (4%)	3 (6%)	
Olfactory epithelium, degeneration			1 (2%)	
Olfactory epithelium, degeneration, hyaline	3 (6%)	2 (4%)	1 (2%)	
Olfactory epithelium, hyperplasia, basal cell		19 (38%)	27 (55%)	26 (52%)
Olfactory epithelium, inflammation, chronic				1 (2%)
Olfactory epithelium, metaplasia	7 (14%)	4 (8%)	5 (10%)	5 (10%)
Olfactory epithelium, necrosis		1 (2%)		1 (2%)
Olfactory epithelium, ulcer			2 (4%)	
Respiratory epithelium, degeneration, hyaline	1 (2%)	1 (2%)		
Respiratory epithelium, hyperplasia		15 (30%)	16 (33%)	23 (46%)
Respiratory epithelium, inflammation, chronic			1 (2%)	2 (4%)
Respiratory epithelium, necrosis				1 (2%)
Squamous epithelium, hyperplasia			1 (2%)	
Squamous epithelium, inflammation			1 (2%)	
Vomeronasal organ, inflammation, suppurative	1 (2%)			
Pleura	(5)	(3)	(5)	(6)
Inflammation, chronic	4 (80%)	3 (100%)	4 (80%)	6 (100%)
Mesothelium, hyperplasia	1 (20%)			
Trachea	(50)	(50)	(50)	(50)
Epithelium, hyperplasia				1 (2%)
Glands, cyst		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(49)	(50)
Degeneration		1 (2%)		
Inflammation, chronic active		1 (2%)		
Bilateral, lens, cataract	1 (2%)	1 (2%)		1 (2%)
Bilateral, retina, atrophy		1 (2%)	1 (2%)	
Ciliary body, iris, inflammation, suppurative			1 (2%)	
Cornea, inflammation, suppurative	2 (4%)			
Cornea, inflammation, chronic				1 (2%)
Lens, cataract	5 (10%)	1 (2%)	2 (4%)	4 (8%)
Retina, atrophy	4 (8%)			2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic	1 (2%)	1 (2%)		
Zymbal's gland	(1)			(2)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Infarct		1 (2%)	2 (4%)	1 (2%)
Infarct, multiple		1 (2%)		
Nephropathy	47 (94%)	47 (94%)	47 (94%)	50 (100%)
Bilateral, renal tubule, cyst			1 (2%)	

TABLE A4

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Urinary System (continued)				
Kidney (continued)	(50)	(50)	(50)	(50)
Bilateral, infarct		1 (2%)		
Capsule, dilatation			1 (2%)	
Glomerulus, inflammation, suppurative		1 (2%)		
Papilla, mineralization	5 (10%)	35 (70%)	44 (88%)	41 (82%)
Pelvis, transitional epithelium, hyperplasia	3 (6%)	5 (10%)	14 (28%)	15 (30%)
Pelvis, dilatation			1 (2%)	1 (2%)
Renal tubule, accumulation, hyaline droplet			1 (2%)	1 (2%)
Renal tubule, cyst		1 (2%)	3 (6%)	2 (4%)
Renal tubule, hyperplasia		3 (6%)	8 (16%)	6 (12%)
Renal tubule, hypertrophy				1 (2%)
Renal tubule, mineralization	1 (2%)			
Ureter		(1)		(1)
Urethra			(1)	(1)
Transitional epithelium, hyperplasia			1 (100%)	
Urinary bladder	(50)	(50)	(49)	(50)
Calculus gross observation			1 (2%)	
Hemorrhage		1 (2%)		
Inflammation, chronic			1 (2%)	
Transitional epithelium, hyperplasia	1 (2%)		4 (8%)	2 (4%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR INHALATION STUDY
OF CUMENE

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TABLE B1

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	23	18	17	15
Natural deaths	6	5	2	3
Survivors				
Terminal sacrifice	21	27	31	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(46)	(45)	(50)	(49)
Intestine large, colon	(48)	(46)	(50)	(50)
Leiomyosarcoma			1 (2%)	
Intestine large, rectum	(48)	(46)	(50)	(50)
Adenoma				1 (2%)
Sarcoma stromal, metastatic, uterus	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Cholangiocarcinoma				1 (2%)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Mesentery	(18)	(18)	(17)	(9)
Rhabdomyosarcoma				1 (11%)
Oral mucosa			(1)	(1)
Squamous cell carcinoma				1 (100%)
Pharyngeal, squamous cell carcinoma			1 (100%)	
Pancreas	(50)	(49)	(50)	(50)
Rhabdomyosarcoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	1 (2%)
Stomach, glandular	(50)	(49)	(50)	(50)
Tongue	(1)	(2)	(2)	(3)
Tooth		(2)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, bone			1 (2%)	
Pericardium, alveolar/bronchiolar, carcinoma, metastatic, lung			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma				1 (2%)
Osteosarcoma, metastatic, bone			1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign	1 (2%)		1 (2%)	
Pheochromocytoma complex	1 (2%)			
Pheochromocytoma malignant	1 (2%)	1 (2%)	1 (2%)	
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Carcinoma	1 (2%)		1 (2%)	
Parathyroid gland	(49)	(45)	(48)	(47)
Pituitary gland	(50)	(50)	(50)	(49)
Adenoma	35 (70%)	33 (66%)	28 (56%)	25 (51%)
Carcinoma	1 (2%)	1 (2%)		1 (2%)

TABLE B1

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
C-cell, adenoma	3 (6%)	4 (8%)	2 (4%)	2 (4%)
C-cell, carcinoma			1 (2%)	2 (4%)
Follicular cell, adenoma		2 (4%)		2 (4%)
Follicular cell, carcinoma			1 (2%)	
General Body System				
Peritoneum			(1)	
Carcinoma, metastatic, ovary			1 (100%)	
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma	1 (2%)	2 (4%)		1 (2%)
Carcinoma, multiple	1 (2%)			
Ovary	(50)	(49)	(50)	(50)
Cystadenocarcinoma			1 (2%)	
Granulosa cell tumor benign		1 (2%)		
Leiomyosarcoma, metastatic, uterus				1 (2%)
Osteosarcoma, metastatic, bone			1 (2%)	
Uterus	(50)	(49)	(50)	(50)
Carcinoma		1 (2%)		
Leiomyosarcoma				1 (2%)
Polyp stromal	13 (26%)	7 (14%)	8 (16%)	7 (14%)
Polyp stromal, multiple		1 (2%)		
Sarcoma stromal	1 (2%)		1 (2%)	1 (2%)
Cervix, polyp stromal		1 (2%)		
Vagina	(2)			
Polyp	1 (50%)			
Epithelium, polyp	1 (50%)			
Hematopoietic System				
Lymph node	(3)	(3)	(5)	(2)
Deep cervical, squamous cell carcinoma, metastatic, skin			1 (20%)	
Lymph node, bronchial	(7)	(9)	(8)	(5)
Lymph node, mandibular	(4)	(1)	(1)	(4)
Carcinoma, metastatic, Zymbal's gland				1 (25%)
Lymph node, mediastinal	(30)	(28)	(26)	(31)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (4%)	
Carcinoma, metastatic, thyroid gland			1 (4%)	2 (6%)
Squamous cell carcinoma, metastatic, skin			1 (4%)	
Lymph node, mesenteric	(48)	(49)	(49)	(50)
Carcinoma, metastatic, ovary			1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Rhabdomyosarcoma				1 (2%)
Thymus	(48)	(46)	(50)	(50)
Thymoma benign				1 (2%)

TABLE B1

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Carcinoma	8 (16%)	8 (16%)	1 (2%)	5 (10%)
Carcinoma, multiple	2 (4%)	1 (2%)	1 (2%)	
Fibroadenoma	17 (34%)	11 (22%)	24 (48%)	21 (42%)
Fibroadenoma, multiple	7 (14%)	11 (22%)	8 (16%)	8 (16%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma			1 (2%)	
Fibrous histiocytoma	1 (2%)			
Keratoacanthoma			2 (4%)	1 (2%)
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma				1 (2%)
Subcutaneous tissue, fibroma		2 (4%)		1 (2%)
Subcutaneous tissue, hemangiopericytoma			1 (2%)	
Subcutaneous tissue, schwannoma benign		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteosarcoma		1 (2%)		
Vertebra, osteosarcoma	1 (2%)		1 (2%)	
Skeletal muscle	(1)		(1)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)	
Fibrous histiocytoma, metastatic, skin	1 (100%)			
Rhabdomyosarcoma				1 (100%)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Astrocytoma malignant				1 (2%)
Carcinoma, metastatic, pituitary gland	1 (2%)	2 (4%)		1 (2%)
Glioma malignant	1 (2%)			
Granular cell tumor benign			1 (2%)	
Meningioma benign		1 (2%)		
Oligodendroglioma malignant	1 (2%)			
Spinal cord		(1)	(1)	(1)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma				1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)		1 (2%)	
Carcinoma, metastatic, ovary			1 (2%)	
Carcinoma, metastatic, uterus		1 (2%)		
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Osteosarcoma, metastatic, bone	1 (2%)		1 (2%)	
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Mediastinum, carcinoma, metastatic, uterus		1 (2%)		
Nose	(50)	(48)	(50)	(50)
Nasopharyngeal duct, carcinoma, metastatic, oral mucosa				1 (2%)
Respiratory epithelium, adenoma		5 (10%)	4 (8%)	3 (6%)
Turbinates, chondroma	1 (2%)			

TABLE B1

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Respiratory System (continued)				
Pleura	(15)	(16)	(16)	(20)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (6%)	
Carcinoma, metastatic, ovary			1 (6%)	
Trachea	(50)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(49)	(50)	(50)
Harderian gland	(50)	(49)	(50)	(50)
Zymbal's gland	(1)		(1)	(1)
Carcinoma	1 (100%)		1 (100%)	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(49)	(50)	(50)
Leiomyosarcoma	1 (2%)			
Systemic Lesions				
Multiple organs ^a	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Leukemia mononuclear	12 (24%)	25 (50%)	23 (46%)	13 (26%)
Lymphoma malignant				1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	50	48	48	47
Total primary neoplasms	117	122	118	111
Total animals with benign neoplasms	44	42	43	43
Total benign neoplasms	81	81	80	78
Total animals with malignant neoplasms	24	32	30	25
Total malignant neoplasms	36	41	38	33
Total animals with metastatic neoplasms	4	3	5	6
Total metastatic neoplasms	8	4	16	6

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate ^a	3/50 (6%)	1/49 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate ^b	7.3%	2.4%	4.7%	0.0%
Terminal rate ^c	1/21 (5%)	1/27 (4%)	2/31 (7%)	0/32 (0%)
First incidence (days)	716	730 (T)	730 (T)	— ^e
Poly-3 test ^d	P=0.096N	P=0.300N	P=0.479N	P=0.109N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	0/50 (0%)	1/50 (2%)
Adjusted rate	7.2%	4.8%	0.0%	2.3%
Terminal rate	1/21 (5%)	1/27 (4%)	0/31 (0%)	1/32 (3%)
First incidence (days)	501	709	—	730 (T)
Poly-3 test	P=0.158N	P=0.502N	P=0.113N	P=0.292N
Mammary Gland: Fibroadenoma				
Overall rate	24/50 (48%)	22/50 (44%)	32/50 (64%)	29/50 (58%)
Adjusted rate	54.9%	49.9%	70.4%	63.4%
Terminal rate	11/21 (52%)	15/27 (56%)	22/31 (71%)	19/32 (59%)
First incidence (days)	501	571	530	619
Poly-3 test	P=0.122	P=0.396N	P=0.087	P=0.267
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	24/50 (48%)	23/50 (46%)	32/50 (64%)	29/50 (58%)
Adjusted rate	54.9%	51.9%	70.4%	63.4%
Terminal rate	11/21 (52%)	15/27 (56%)	22/31 (71%)	19/32 (59%)
First incidence (days)	501	571	530	619
Poly-3 test	P=0.138	P=0.472N	P=0.087	P=0.267
Mammary Gland: Carcinoma				
Overall rate	10/50 (20%)	9/50 (18%)	2/50 (4%)	5/50 (10%)
Adjusted rate	23.9%	21.2%	4.7%	11.2%
Terminal rate	5/21 (24%)	8/27 (30%)	1/31 (3%)	1/32 (3%)
First incidence (days)	558	704	715	542
Poly-3 test	P=0.038N	P=0.484N	P=0.011N	P=0.101N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	10/50 (20%)	2/50 (4%)	6/50 (12%)
Adjusted rate	23.9%	23.4%	4.7%	13.4%
Terminal rate	5/21 (24%)	8/27 (30%)	1/31 (3%)	1/32 (3%)
First incidence (days)	558	673	715	542
Poly-3 test	P=0.063N	P=0.580N	P=0.011N	P=0.162N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	31/50 (62%)	29/50 (58%)	32/50 (64%)	33/50 (66%)
Adjusted rate	69.9%	65.3%	70.4%	70.5%
Terminal rate	16/21 (76%)	20/27 (74%)	22/31 (71%)	20/32 (63%)
First incidence (days)	501	571	530	542
Poly-3 test	P=0.444	P=0.400N	P=0.576	P=0.570
Nose: Adenoma				
Overall rate	0/50 (0%)	5/48 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	0.0%	12.2%	9.3%	6.9%
Terminal rate	0/21 (0%)	5/27 (19%)	4/31 (13%)	2/32 (6%)
First incidence (days)	—	730 (T)	730 (T)	638
Poly-3 test	P=0.320	P=0.030	P=0.066	P=0.130

TABLE B2

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	35/50 (70%)	33/50 (66%)	28/50 (56%)	25/49 (51%)
Adjusted rate	77.1%	71.4%	61.4%	56.2%
Terminal rate	17/21 (81%)	17/27 (63%)	19/31 (61%)	17/32 (53%)
First incidence (days)	501	536	528	542
Poly-3 test	P=0.013N	P=0.342N	P=0.070N	P=0.023N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	36/50 (72%)	34/50 (68%)	28/50 (56%)	26/49 (53%)
Adjusted rate	78.9%	72.7%	61.4%	58.1%
Terminal rate	17/21 (81%)	17/27 (63%)	19/31 (61%)	17/32 (53%)
First incidence (days)	501	536	528	542
Poly-3 test	P=0.012N	P=0.319N	P=0.045N	P=0.022N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	6.8%	4.6%
Terminal rate	0/21 (0%)	0/27 (0%)	1/31 (3%)	1/32 (3%)
First incidence (days)	—	—	516	683
Poly-3 test	P=0.106	—	P=0.132	P=0.250
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	9.1%	4.6%
Terminal rate	0/21 (0%)	0/27 (0%)	2/31 (7%)	1/32 (3%)
First incidence (days)	—	— ^f	516	683
Poly-3 test	P=0.114	— ^f	P=0.069	P=0.250
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	4/49 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	7.3%	9.5%	4.7%	4.6%
Terminal rate	2/21 (10%)	2/27 (7%)	2/31 (7%)	2/32 (6%)
First incidence (days)	716	655	730 (T)	730 (T)
Poly-3 test	P=0.295N	P=0.513	P=0.479N	P=0.476N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	4/49 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	7.3%	9.5%	7.0%	9.3%
Terminal rate	2/21 (10%)	2/27 (7%)	3/31 (10%)	4/32 (13%)
First incidence (days)	716	655	730 (T)	730 (T)
Poly-3 test	P=0.495	P=0.513	P=0.641N	P=0.529
Uterus: Stromal Polyp				
Overall rate	13/50 (26%)	9/50 (18%)	8/50 (16%)	7/50 (14%)
Adjusted rate	31.1%	21.1%	18.2%	15.9%
Terminal rate	10/21 (48%)	7/27 (26%)	6/31 (19%)	4/32 (13%)
First incidence (days)	547	696	528	628
Poly-3 test	P=0.070N	P=0.210N	P=0.125N	P=0.076N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	14/50 (28%)	9/50 (18%)	9/50 (18%)	8/50 (16%)
Adjusted rate	33.5%	21.1%	20.3%	17.9%
Terminal rate	10/21 (48%)	7/27 (26%)	6/31 (19%)	4/32 (13%)
First incidence (days)	547	696	528	411
Poly-3 test	P=0.081N	P=0.148N	P=0.124N	P=0.075N

TABLE B2

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
All Organs: Mononuclear Leukemia				
Overall rate	12/50 (24%)	25/50 (50%)	23/50 (46%)	13/50 (26%)
Adjusted rate	27.9%	55.5%	50.2%	29.0%
Terminal rate	3/21 (14%)	14/27 (52%)	12/31 (39%)	7/32 (22%)
First incidence (days)	492	547	530	542
Poly-3 test	P=0.277N	P=0.006	P=0.023	P=0.548
All Organs: Benign Neoplasms				
Overall rate	44/50 (88%)	42/50 (84%)	43/50 (86%)	43/50 (86%)
Adjusted rate	93.2%	89.8%	89.7%	91.8%
Terminal rate	21/21 (100%)	24/27 (89%)	28/31 (90%)	30/32 (94%)
First incidence (days)	374	536	516	542
Poly-3 test	P=0.519N	P=0.399N	P=0.390N	P=0.559N
All Organs: Malignant Neoplasms				
Overall rate	24/50 (48%)	33/50 (66%)	30/50 (60%)	25/50 (50%)
Adjusted rate	53.0%	70.8%	62.1%	52.7%
Terminal rate	7/21 (33%)	19/27 (70%)	15/31 (48%)	13/32 (41%)
First incidence (days)	485	514	339	267
Poly-3 test	P=0.304N	P=0.053	P=0.242	P=0.573N
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	48/50 (96%)	48/50 (96%)	47/50 (94%)
Adjusted rate	100%	98.0%	96.0%	95.6%
Terminal rate	21/21 (100%)	26/27 (96%)	29/31 (94%)	30/32 (94%)
First incidence (days)	374	514	339	267
Poly-3 test	P=0.132N	P=0.496N	P=0.237N	P=0.200N

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, nose, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

^b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by N.

^e Not applicable; no neoplasms in animal group

^f Value of statistic cannot be computed.

TABLE B3

Historical Incidence of Adenoma of the Nose in Control Female F344/N Rats^a

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	0/50
Decalin	0/50
Divinylbenzene	0/50
Methyl isobutyl ketone	0/50
α -Methylstyrene	0/49
Propargyl alcohol	0/49
Propylene glycol mono- <i>t</i> -butyl ether	0/49
Stoddard solvent IIC	0/49
Vanadium pentoxide	0/50
Overall Historical Incidence: Inhalation Studies	
Total	0/496
Overall Historical Incidence: All Routes	
Total (%)	0/1,343

^a Data as of March 2, 2007

TABLE B4

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	23	18	17	15
Natural deaths	6	5	2	3
Survivors				
Terminal sacrifice	21	27	31	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(46)	(45)	(50)	(49)
Necrosis				1 (2%)
Intestine large, colon	(48)	(46)	(50)	(50)
Artery, inflammation, chronic				1 (2%)
Intestine large, rectum	(48)	(46)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		2 (4%)	
Basophilic focus	27 (54%)	31 (62%)	36 (72%)	32 (64%)
Clear cell focus	15 (30%)	9 (18%)	6 (12%)	5 (10%)
Eosinophilic focus				1 (2%)
Hepatodiaphragmatic nodule	6 (12%)	6 (12%)	4 (8%)	6 (12%)
Inflammation, granulomatous	1 (2%)			
Mixed cell focus			2 (4%)	1 (2%)
Necrosis		1 (2%)	1 (2%)	3 (6%)
Vacuolization cytoplasmic	11 (22%)	4 (8%)	4 (8%)	2 (4%)
Bile duct, hyperplasia			1 (2%)	
Centrilobular, congestion		1 (2%)		
Periportal, vacuolization cytoplasmic	1 (2%)			
Mesentery	(18)	(18)	(17)	(9)
Necrosis	18 (100%)	16 (89%)	17 (100%)	8 (89%)
Oral mucosa			(1)	(1)
Pancreas	(50)	(49)	(50)	(50)
Fibrosis		1 (2%)		
Hemorrhage		1 (2%)		
Thrombosis				1 (2%)
Artery, inflammation, chronic				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion			1 (2%)	
Inflammation, suppurative				1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Ulcer	5 (10%)	4 (8%)	3 (6%)	1 (2%)
Epithelium, cyst			1 (2%)	
Epithelium, erosion		1 (2%)		
Epithelium, hyperplasia	11 (22%)	13 (26%)	12 (24%)	8 (16%)
Submucosa, fibrosis			1 (2%)	
Stomach, glandular	(50)	(49)	(50)	(50)
Erosion	1 (2%)			
Ulcer			1 (2%)	
Epithelium, hyperplasia				1 (2%)
Tongue	(1)	(2)	(2)	(3)
Epithelium, hyperplasia	1 (100%)	2 (100%)	2 (100%)	3 (100%)
Tooth		(2)		
Peridontal tissue, inflammation		2 (100%)		

^a Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	1 (2%)	2 (4%)	2 (4%)	
Inflammation, chronic				1 (2%)
Atrium, thrombosis		2 (4%)	2 (4%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Hemorrhage	1 (2%)			
Hyperplasia	9 (18%)	9 (18%)	13 (26%)	12 (24%)
Necrosis	2 (4%)			1 (2%)
Vacuolization cytoplasmic	11 (22%)	18 (37%)	16 (32%)	7 (14%)
Adrenal medulla	(50)	(49)	(50)	(50)
Islets, pancreatic	(50)	(49)	(50)	(50)
Parathyroid gland	(49)	(45)	(48)	(47)
Hyperplasia		1 (2%)		
Pituitary gland	(50)	(50)	(50)	(49)
Cyst	7 (14%)	2 (4%)	2 (4%)	2 (4%)
Hemorrhage	2 (4%)	2 (4%)	2 (4%)	
Hyperplasia	10 (20%)	13 (26%)	6 (12%)	16 (33%)
Thyroid gland	(50)	(49)	(50)	(50)
Ultimobranchial cyst	1 (2%)			
C-cell, hyperplasia	13 (26%)	13 (27%)	15 (30%)	15 (30%)
Follicular cell, hyperplasia			1 (2%)	
General Body System				
Peritoneum			(1)	
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Cyst		2 (4%)	2 (4%)	1 (2%)
Hyperplasia	2 (4%)	5 (10%)	7 (14%)	5 (10%)
Inflammation, chronic		1 (2%)	1 (2%)	3 (6%)
Ovary	(50)	(49)	(50)	(50)
Cyst	3 (6%)	7 (14%)	7 (14%)	6 (12%)
Cyst, multiple			1 (2%)	
Uterus	(50)	(49)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Pigmentation			1 (2%)	
Endometrium, hyperplasia	4 (8%)	7 (14%)	2 (4%)	5 (10%)
Vagina	(2)			
Epithelium, hyperplasia, adenomatous	1 (50%)			
Hematopoietic System				
Lymph node	(3)	(3)	(5)	(2)
Pancreatic, infiltration cellular, histiocyte			1 (20%)	
Pancreatic, pigmentation			1 (20%)	
Lymph node, bronchial	(7)	(9)	(8)	(5)
Ectasia	2 (29%)			
Hemorrhage				1 (20%)
Hyperplasia, lymphoid		1 (11%)		1 (20%)
Inflammation	1 (14%)			

TABLE B4

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Hematopoietic System (continued)				
Lymph node, mandibular	(4)	(1)	(1)	(4)
Ectasia				1 (25%)
Hyperplasia, lymphoid				1 (25%)
Lymph node, mediastinal	(30)	(28)	(26)	(31)
Angiectasis			1 (4%)	
Hemorrhage			1 (4%)	1 (3%)
Hyperplasia, histiocytic			1 (4%)	
Hyperplasia, lymphoid			1 (4%)	2 (6%)
Inflammation, suppurative				1 (3%)
Lymph node, mesenteric	(48)	(49)	(49)	(50)
Ectasia			1 (2%)	
Hemorrhage	2 (4%)			
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, histiocyte		1 (2%)		
Necrosis				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Fibrosis			3 (6%)	
Hematopoietic cell proliferation		1 (2%)	2 (4%)	2 (4%)
Necrosis				2 (4%)
Capsule, fibrosis	1 (2%)			
Thymus	(48)	(46)	(50)	(50)
Hyperplasia, tubular			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	3 (6%)			2 (4%)
Hyperplasia	1 (2%)			
Inflammation, suppurative		1 (2%)		
Epithelium, hyperplasia			1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Hyperkeratosis	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic				1 (2%)
Ulcer		3 (6%)		2 (4%)
Sebaceous gland, hemorrhage		1 (2%)		
Subcutaneous tissue, fibrosis				1 (2%)
Subcutaneous tissue, inflammation, suppurative		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		1 (2%)		
Maxilla, fracture			1 (2%)	
Maxilla, inflammation, chronic active				1 (2%)
Skeletal muscle	(1)		(1)	(1)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Compression	12 (24%)	9 (18%)	4 (8%)	5 (10%)
Demyelination				1 (2%)
Gliosis				1 (2%)
Hemorrhage	1 (2%)	3 (6%)	2 (4%)	3 (6%)

TABLE B4

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Nervous System (continued)				
Brain (continued)	(50)	(49)	(50)	(50)
Inflammation, chronic active				1 (2%)
Necrosis	1 (2%)			
Thrombosis		1 (2%)	1 (2%)	
Medulla, meninges, inflammation, suppurative	1 (2%)			
Medulla, neuron, necrosis				1 (2%)
Neuron, degeneration				1 (2%)
Spinal cord		(1)	(1)	(1)
Gliosis				1 (100%)
Inflammation, chronic active				1 (100%)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Foreign body	2 (4%)	1 (2%)	2 (4%)	
Inflammation, suppurative	3 (6%)	3 (6%)	4 (8%)	1 (2%)
Inflammation, chronic	2 (4%)		2 (4%)	2 (4%)
Respiratory epithelium, hyperplasia		1 (2%)		1 (2%)
Lung	(50)	(50)	(50)	(50)
Cyst, squamous				1 (2%)
Hemorrhage	2 (4%)	1 (2%)	2 (4%)	
Inflammation, suppurative	1 (2%)			
Inflammation, chronic	11 (22%)	10 (20%)	8 (16%)	16 (32%)
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)	5 (10%)	7 (14%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Alveolus, infiltration cellular, histiocyte	16 (32%)	6 (12%)	11 (22%)	14 (28%)
Alveolus, proteinosis				2 (4%)
Bronchiole, hyperplasia		3 (6%)		1 (2%)
Bronchiole, inflammation, chronic	1 (2%)	1 (2%)		3 (6%)
Interstitial, fibrosis	1 (2%)			
Nose	(50)	(48)	(50)	(50)
Foreign body		4 (8%)	2 (4%)	3 (6%)
Hyperplasia, basal cell				1 (2%)
Inflammation, suppurative	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Inflammation, chronic	1 (2%)	3 (6%)	4 (8%)	1 (2%)
Inflammation, chronic active			1 (2%)	
Glands, respiratory epithelium, dilatation	1 (2%)			
Goblet cell, hyperplasia	4 (8%)	6 (13%)	1 (2%)	5 (10%)
Nasolacrimal duct, inflammation, suppurative	4 (8%)	3 (6%)	2 (4%)	1 (2%)
Nasolacrimal duct, inflammation, chronic	1 (2%)			
Olfactory epithelium, degeneration, hyaline	2 (4%)	1 (2%)		
Olfactory epithelium, hyperplasia			1 (2%)	
Olfactory epithelium, hyperplasia, basal cell		14 (29%)	25 (50%)	31 (62%)
Olfactory epithelium, inflammation, granulomatous		1 (2%)		
Olfactory epithelium, metaplasia		1 (2%)		2 (4%)
Olfactory epithelium, necrosis			1 (2%)	
Respiratory epithelium, degeneration, hyaline	1 (2%)		3 (6%)	
Respiratory epithelium, hyperplasia			4 (8%)	6 (12%)
Respiratory epithelium, metaplasia, squamous		1 (2%)		
Turbinate, necrosis			1 (2%)	
Pleura	(15)	(16)	(16)	(20)
Inflammation, chronic	15 (100%)	15 (94%)	14 (88%)	20 (100%)
Mesothelium, hyperplasia		1 (6%)		
Trachea	(50)	(49)	(50)	(50)
Glands, cyst			1 (2%)	

TABLE B4

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Special Senses System				
Eye	(50)	(49)	(50)	(50)
Bilateral, lens, cataract	1 (2%)			
Bilateral, retina, atrophy	1 (2%)	1 (2%)	1 (2%)	
Cornea, epithelium, hyperplasia				1 (2%)
Cornea, inflammation				1 (2%)
Cornea, inflammation, suppurative	1 (2%)			
Lens, cataract	5 (10%)	2 (4%)	5 (10%)	3 (6%)
Retina, atrophy	3 (6%)	5 (10%)	8 (16%)	2 (4%)
Harderian gland	(50)	(49)	(50)	(50)
Inflammation, chronic	1 (2%)		1 (2%)	1 (2%)
Zymbal's gland	(1)		(1)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct, multiple			1 (2%)	
Nephropathy	38 (76%)	37 (74%)	41 (82%)	44 (88%)
Artery, inflammation, chronic active				1 (2%)
Papilla, mineralization	6 (12%)	3 (6%)	4 (8%)	6 (12%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)	1 (2%)	6 (12%)	1 (2%)
Pelvis, transitional epithelium, mineralization	23 (46%)	27 (54%)	27 (54%)	22 (44%)
Pelvis, dilatation	1 (2%)			
Renal tubule, accumulation, hyaline droplet	1 (2%)		1 (2%)	
Renal tubule, cyst	1 (2%)		1 (2%)	1 (2%)
Renal tubule, pigmentation	1 (2%)			
Urinary bladder	(50)	(49)	(50)	(50)
Transitional epithelium, hyperplasia	1 (2%)	2 (4%)	2 (4%)	1 (2%)

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

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TABLE C1

Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	9	13	20
Natural deaths	5	7	7	7
Survivors				
Terminal sacrifice	38	34	30	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(43)	(32)	(37)	(38)
Intestine large, cecum	(49)	(47)	(44)	(44)
Mast cell tumor malignant, metastatic, uncertain primary site		1 (2%)		
Intestine large, colon	(50)	(46)	(45)	(48)
Leiomyoma		1 (2%)		
Intestine small, duodenum	(49)	(44)	(43)	(44)
Carcinoma		1 (2%)		
Intestine small, ileum	(49)	(46)	(43)	(44)
Intestine small, jejunum	(48)	(46)	(43)	(44)
Carcinoma	1 (2%)	1 (2%)		2 (5%)
Liver	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Hemangiosarcoma		1 (2%)	1 (2%)	1 (2%)
Hepatoblastoma	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Hepatocellular adenoma	17 (34%)	13 (26%)	15 (30%)	9 (18%)
Hepatocellular adenoma, multiple	17 (34%)	20 (40%)	22 (44%)	26 (52%)
Hepatocellular carcinoma	10 (20%)	17 (34%)	17 (34%)	10 (20%)
Hepatocellular carcinoma, multiple	3 (6%)	1 (2%)	4 (8%)	7 (14%)
Hepatocholangiocarcinoma				1 (2%)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Mesentery	(3)	(7)	(6)	(3)
Mast cell tumor malignant, metastatic, uncertain primary site		1 (14%)		
Pancreas	(50)	(50)	(49)	(49)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(49)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (2%)	1 (2%)		
Sarcoma, metastatic, uncertain primary site				1 (2%)
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(50)	(48)	(48)	(48)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Tongue	(1)			
Squamous cell carcinoma	1 (100%)			
Tooth	(15)	(8)	(8)	(6)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (7%)	1 (13%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Hemangiosarcoma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Sarcoma, metastatic, uncertain primary site				1 (2%)
Subcapsular, adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Adrenal medulla	(50)	(50)	(48)	(50)
Pheochromocytoma benign		1 (2%)	1 (2%)	
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(50)	(50)	(49)	(49)
Pituitary gland	(48)	(49)	(49)	(49)
Pars distalis, adenoma	1 (2%)			1 (2%)
Thyroid gland	(50)	(50)	(49)	(50)
Follicular cell, adenoma				3 (6%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(49)	(50)
Preputial gland	(49)	(50)	(50)	(50)
Prostate	(50)	(49)	(48)	(49)
Testes	(50)	(50)	(49)	(50)
Interstitial cell, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(49)
Lymph node			(1)	(2)
Pancreatic, sarcoma, metastatic, uncertain primary site				1 (50%)
Renal, carcinoma, metastatic, uncertain primary site				1 (50%)
Lymph node, bronchial	(45)	(41)	(45)	(35)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (3%)
Carcinoma, metastatic, uncertain primary site				1 (3%)
Sarcoma, metastatic, uncertain primary site				1 (3%)
Lymph node, mandibular	(34)	(35)	(22)	(25)
Carcinoma, metastatic, uncertain primary site				1 (4%)
Mast cell tumor metastatic, uncertain primary site	1 (3%)			
Lymph node, mediastinal	(37)	(42)	(40)	(39)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (3%)	2 (5%)
Carcinoma, metastatic, uncertain primary site				1 (3%)
Hepatocellular carcinoma, metastatic, liver				1 (3%)
Sarcoma, metastatic, uncertain primary site				1 (3%)
Lymph node, mesenteric	(48)	(47)	(46)	(44)
Sarcoma, metastatic, uncertain primary site				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Hematopoietic System (continued)				
Spleen	(50)	(50)	(49)	(50)
Hemangiosarcoma				4 (8%)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Thymus	(45)	(44)	(38)	(44)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (2%)			
Sarcoma, metastatic, uncertain primary site				1 (2%)
Integumentary System				
Skin	(50)	(50)	(49)	(50)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Skeletal muscle			(1)	
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	12 (24%)	19 (38%)	16 (32%)	9 (18%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	12 (24%)	15 (30%)	20 (40%)
Alveolar/bronchiolar carcinoma	9 (18%)	11 (22%)	12 (24%)	16 (32%)
Alveolar/bronchiolar carcinoma, multiple		8 (16%)	20 (40%)	17 (34%)
Carcinoma, metastatic, Harderian gland	1 (2%)		2 (4%)	1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Hepatocellular carcinoma, metastatic, liver	7 (14%)	8 (16%)	11 (22%)	7 (14%)
Sarcoma, metastatic, uncertain primary site			1 (2%)	1 (2%)
Nose	(50)	(50)	(49)	(48)
Pleura				(2)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (50%)
Carcinoma, metastatic, uncertain primary site				1 (50%)
Trachea	(50)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Special Senses System				
Eye	(49)	(50)	(49)	(48)
Harderian gland	(48)	(50)	(49)	(50)
Adenoma	8 (17%)	2 (4%)	3 (6%)	4 (8%)
Carcinoma	4 (8%)	3 (6%)	2 (4%)	2 (4%)
Bilateral, adenoma			1 (2%)	1 (2%)

TABLE C1

Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	1 (2%)		
Mast cell tumor malignant, metastatic, uncertain primary site	1 (2%)	1 (2%)		
Capsule, sarcoma, metastatic, uncertain primary site				1 (2%)
Urinary bladder	(50)	(48)	(49)	(48)
Hemangiosarcoma			1 (2%)	
Systemic Lesions				
Multiple organs ^b	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant	2 (4%)	1 (2%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms ^c	48	50	50	50
Total primary neoplasms	93	119	140	140
Total animals with benign neoplasms	43	45	46	44
Total benign neoplasms	60	69	75	74
Total animals with malignant neoplasms	26	34	47	45
Total malignant neoplasms	33	50	65	66
Total animals with metastatic neoplasms	9	10	15	12
Total metastatic neoplasms	14	16	19	40
Total animals with malignant neoplasms-uncertain primary site	1	1	1	2

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2

Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Harderian Gland: Adenoma				
Overall rate ^a	8/50 (16%)	2/50 (4%)	4/50 (8%)	5/50 (10%)
Adjusted rate ^b	17.2%	4.5%	9.0%	12.2%
Terminal rate ^c	8/38 (21%)	2/34 (6%)	3/30 (10%)	2/23 (9%)
First incidence (days) ^d	729 (T)	729 (T)	565	556
Poly-3 test	P=0.400N	P=0.052N	P=0.195N	P=0.358N
Harderian Gland: Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	8.5%	6.7%	4.5%	5.0%
Terminal rate	3/38 (8%)	3/34 (9%)	2/30 (7%)	1/23 (4%)
First incidence (days)	551	729 (T)	729 (T)	705
Poly-3 test	P=0.297N	P=0.529N	P=0.367N	P=0.412N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	11/50 (22%)	5/50 (10%)	6/50 (12%)	7/50 (14%)
Adjusted rate	23.4%	11.2%	13.4%	17.0%
Terminal rate	10/38 (26%)	5/34 (15%)	5/30 (17%)	3/23 (13%)
First incidence (days)	551	729 (T)	565	556
Poly-3 test	P=0.322N	P=0.102N	P=0.168N	P=0.315N
Liver: Hepatocellular Adenoma				
Overall rate	34/50 (68%)	33/50 (66%)	37/50 (74%)	35/50 (70%)
Adjusted rate	70.6%	69.9%	77.9%	79.5%
Terminal rate	28/38 (74%)	23/34 (68%)	25/30 (83%)	20/23 (87%)
First incidence (days)	551	533	381	526
Poly-3 test	P=0.135	P=0.560N	P=0.276	P=0.218
Liver: Hepatocellular Carcinoma				
Overall rate	13/50 (26%)	18/50 (36%)	21/50 (42%)	17/50 (34%)
Adjusted rate	27.1%	38.1%	43.3%	37.8%
Terminal rate	7/38 (18%)	9/34 (27%)	7/30 (23%)	4/23 (17%)
First incidence (days)	583	453	381	391
Poly-3 test	P=0.184	P=0.177	P=0.071	P=0.190
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	40/50 (80%)	42/50 (84%)	43/50 (86%)	41/50 (82%)
Adjusted rate	81.0%	85.8%	87.2%	87.1%
Terminal rate	30/38 (79%)	28/34 (82%)	26/30 (87%)	20/23 (87%)
First incidence (days)	551	453	381	391
Poly-3 test	P=0.250	P=0.355	P=0.284	P=0.286
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.1%	2.2%	9.0%	7.4%
Terminal rate	0/38 (0%)	1/34 (3%)	3/30 (10%)	2/23 (9%)
First incidence (days)	659	729 (T)	702	610
Poly-3 test	P=0.118	P=0.750	P=0.163	P=0.256
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	14/50 (28%)	18/50 (36%)	24/50 (48%)	20/50 (40%)
Adjusted rate	29.1%	38.1%	49.4%	44.0%
Terminal rate	7/38 (18%)	9/34 (27%)	9/30 (30%)	6/23 (26%)
First incidence (days)	583	453	381	391
Poly-3 test	P=0.071	P=0.236	P=0.030	P=0.096

TABLE C2

Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	41/50 (82%)	42/50 (84%)	44/50 (88%)	42/50 (84%)
Adjusted rate	82.6%	85.8%	89.0%	88.5%
Terminal rate	30/38 (79%)	28/34 (82%)	26/30 (87%)	20/23 (87%)
First incidence (days)	551	453	381	391
Poly-3 test	P=0.227	P=0.437	P=0.264	P=0.291
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	13/50 (26%)	31/50 (62%)	31/50 (62%)	29/50 (58%)
Adjusted rate	27.5%	66.7%	66.9%	67.9%
Terminal rate	10/38 (26%)	25/34 (74%)	23/30 (77%)	20/23 (87%)
First incidence (days)	628	551	512	480
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	9/50 (18%)	19/50 (38%)	32/50 (64%)	33/50 (66%)
Adjusted rate	19.1%	41.5%	70.5%	71.3%
Terminal rate	6/38 (16%)	15/34 (44%)	25/30 (83%)	12/23 (52%)
First incidence (days)	631	551	565	420
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	19/50 (38%)	38/50 (76%)	42/50 (84%)	43/50 (86%)
Adjusted rate	39.8%	81.4%	89.5%	92.1%
Terminal rate	14/38 (37%)	31/34 (91%)	30/30 (100%)	21/23 (91%)
First incidence (days)	628	551	512	420
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Skin: Fibrous Histiocytoma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	4.5%	2.3%	7.4%
Terminal rate	1/38 (3%)	2/34 (6%)	1/30 (3%)	2/23 (9%)
First incidence (days)	729 (T)	729 (T)	729 (T)	609
Poly-3 test	P=0.199	P=0.486	P=0.749	P=0.258
Skin: Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.3%	6.7%	2.3%	7.4%
Terminal rate	1/38 (3%)	2/34 (6%)	1/30 (3%)	2/23 (9%)
First incidence (days)	583	652	729 (T)	609
Poly-3 test	P=0.416	P=0.479	P=0.521N	P=0.433
Spleen: Hemangiosarcoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/49 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	9.9%
Terminal rate	0/38 (0%)	0/34 (0%)	0/30 (0%)	3/23 (13%)
First incidence (days)	— ^e	— ^f	—	556
Poly-3 test	P=0.002	— ^f	—	P=0.045
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/49 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	7.5%
Terminal rate	0/38 (0%)	0/34 (0%)	0/30 (0%)	2/23 (9%)
First incidence (days)	—	—	—	680
Poly-3 test	P=0.010	—	—	P=0.095

TABLE C2

Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	2.2%	4.5%	9.9%
Terminal rate	0/38 (0%)	0/34 (0%)	2/30 (7%)	3/23 (13%)
First incidence (days)	—	654	729 (T)	556
Poly-3 test	P=0.015	P=0.493	P=0.226	P=0.045
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.2%	2.2%	4.5%	9.9%
Terminal rate	1/38 (3%)	0/34 (0%)	2/30 (7%)	3/23 (13%)
First incidence (days)	729 (T)	654	729 (T)	556
Poly-3 test	P=0.054	P=0.753	P=0.482	P=0.141
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	45/50 (90%)	46/50 (92%)	44/50 (88%)
Adjusted rate	87.8%	93.2%	94.5%	95.6%
Terminal rate	34/38 (90%)	32/34 (94%)	30/30 (100%)	23/23 (100%)
First incidence (days)	551	533	381	480
Poly-3 test	P=0.091	P=0.275	P=0.192	P=0.132
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	34/50 (68%)	47/50 (94%)	45/50 (90%)
Adjusted rate	55.0%	69.8%	95.2%	90.8%
Terminal rate	18/38 (47%)	21/34 (62%)	28/30 (93%)	19/23 (83%)
First incidence (days)	551	453	381	391
Poly-3 test	P<0.001	P=0.094	P<0.001	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	96.0%	100%	100%	100%
Terminal rate	36/38 (95%)	34/34 (100%)	30/30 (100%)	23/23 (100%)
First incidence (days)	551	453	381	391
Poly-3 test	P=0.114	P=0.237	P=0.237	P=0.237

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, spleen, and thyroid gland; for other tissues, denominator is number of animals necropsied.

^b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by N.

^e Not applicable; no neoplasms in animal group

^f Value of statistic cannot be computed.

TABLE C3a

Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F1 Mice^a

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
Cumene	13/50	9/50	19/50
Decalin	8/50	8/50	15/50
Divinylbenzene	12/49	5/49	16/49
Methyl isobutyl ketone	9/50	5/50	14/50
α -Methylstyrene	8/50	5/50	13/50
Propargyl alcohol	10/50	7/50	17/50
Propylene glycol mono- <i>t</i> -butyl ether	13/50	6/50	17/50
Stoddard solvent IIC	6/50	7/50	13/50
Vanadium pentoxide	13/50	12/50	22/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	92/449 (20.5%)	64/449 (14.3%)	146/449 (32.5%)
Mean \pm standard deviation	20.5% \pm 5.3%	14.2% \pm 4.6%	32.5% \pm 5.9%
Range	12%-26%	10%-24%	26%-44%
Overall Historical Incidence: All Routes			
Total (%)	263/1,498 (17.6%)	161/1,498 (10.8%)	401/1,498 (26.8%)
Mean \pm standard deviation	17.9% \pm 6.1%	10.9% \pm 5.6%	27.2% \pm 7.8%
Range	6%-28%	2%-24%	12%-44%

^a Data as of March 2, 2007

TABLE C3b
Historical Incidence of Hepatocellular Neoplasms in Control Male B6C3F1 Mice^a

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
Cumene	34/50	13/50	40/50
Decalin	22/50	10/50	28/50
Divinylbenzene	22/50	13/50	30/50
Methyl isobutyl ketone	17/50	12/50	27/50
α -Methylstyrene	24/50	10/50	28/50
Propargyl alcohol	21/49	10/49	26/49
Propylene glycol mono- <i>t</i> -butyl ether	18/50	9/50	25/50
Stoddard solvent IIC	23/50	16/50	34/50
Vanadium pentoxide	15/50	14/50	26/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	196/449 (43.7%)	107/449 (23.8%)	264/449 (58.8%)
Mean \pm standard deviation	43.7% \pm 10.9%	23.8% \pm 4.6%	58.8% \pm 9.6%
Range	30%-68%	18%-32%	50%-80%
Overall Historical Incidence: All Routes			
Total (%)	633/1,496 (42.3%)	382/1,496 (25.5%)	874/1,496 (58.4%)
Mean \pm standard deviation	43.3% \pm 14.2%	26.0% \pm 9.1%	59.6% \pm 15.4%
Range	14%-70%	8%-48%	20%-85%

^a Data as of March 2, 2007

TABLE C3c
Historical Incidence of Hemangiosarcoma in Control Male B6C3F1 Mice^a

Study	Incidence in Controls	
	Spleen	All Organs
Historical Incidence: Inhalation Studies		
Cumene	0/50	0/50
Decalin	0/49	1/50
Divinylbenzene	1/49	6/50
Methyl isobutyl ketone	1/50	3/50
α -Methylstyrene	1/50	4/50
Propargyl alcohol	1/49	2/50
Propylene glycol mono- <i>t</i> -butyl ether	2/48	3/50
Stoddard solvent IIC	0/49	1/50
Vanadium pentoxide	0/50	1/50
Overall Historical Incidence: Inhalation Studies		
Total (%)	6/444 (1.4%)	21/450 (4.7%)
Mean \pm standard deviation	1.4% \pm 1.5%	4.7% \pm 3.7%
Range	0%-4%	0%-12%
Overall Historical Incidence: All Routes		
Total (%)	24/1,483 (1.6%)	76/1,499 (5.1%)
Mean \pm standard deviation	1.7% \pm 1.2%	5.2% \pm 3.2%
Range	0%-4%	0%-12%

^a Data as of March 2, 2007

TABLE C3d

Historical Incidence of Follicular Cell Adenoma of the Thyroid Gland in Control Male B6C3F1 Mice^a

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	0/50
Decalin	0/48
Divinylbenzene	0/49
Methyl isobutyl ketone	0/50
α -Methylstyrene	3/50
Propargyl alcohol	0/49
Propylene glycol mono- <i>t</i> -butyl ether	1/48
Stoddard solvent IIC	1/49
Vanadium pentoxide	0/48
Overall Historical Incidence: Inhalation Studies	
Total (%)	5/441 (1.1%)
Mean \pm standard deviation	1.1% \pm 2.0%
Range	0%-6%
Overall Historical Incidence: All Routes	
Total (%)	21/1,483 (1.4%)
Mean \pm standard deviation	1.4% \pm 1.8%
Range	0%-6%

^a Data as of March 2, 2007

TABLE C4

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	9	13	20
Natural deaths	5	7	7	7
Survivors				
Terminal sacrifice	38	34	30	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(43)	(32)	(37)	(38)
Degeneration, hyaline	1 (2%)			
Hyperplasia	1 (2%)			
Intestine large, cecum	(49)	(47)	(44)	(44)
Infiltration cellular, histiocyte				1 (2%)
Inflammation, acute				1 (2%)
Intestine large, colon	(50)	(46)	(45)	(48)
Intestine small, duodenum	(49)	(44)	(43)	(44)
Intestine small, ileum	(49)	(46)	(43)	(44)
Necrosis			1 (2%)	
Peyer's patch, inflammation, acute	1 (2%)			
Intestine small, jejunum	(48)	(46)	(43)	(44)
Peyer's patch, hyperplasia		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	3 (6%)	1 (2%)	
Basophilic focus	7 (14%)	5 (10%)	4 (8%)	5 (10%)
Clear cell focus	24 (48%)	11 (22%)	20 (40%)	11 (22%)
Eosinophilic focus	6 (12%)	5 (10%)	16 (32%)	14 (28%)
Hepatodiaphragmatic nodule				1 (2%)
Inflammation, granulomatous	1 (2%)		1 (2%)	
Mixed cell focus	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Necrosis	2 (4%)	4 (8%)	4 (8%)	1 (2%)
Tension lipidosis	1 (2%)	1 (2%)	2 (4%)	
Centrilobular, necrosis	1 (2%)	4 (8%)		
Mesentery	(3)	(7)	(6)	(3)
Thrombosis	1 (33%)			
Fat, necrosis	3 (100%)	6 (86%)	6 (100%)	3 (100%)
Pancreas	(50)	(50)	(49)	(49)
Atrophy		1 (2%)		
Duct, cyst	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(49)
Inflammation		2 (4%)	1 (2%)	5 (10%)
Ulcer	1 (2%)	4 (8%)	6 (12%)	6 (12%)
Epithelium, erosion		1 (2%)		1 (2%)
Epithelium, hyperplasia	2 (4%)	7 (14%)	8 (16%)	13 (27%)
Stomach, glandular	(50)	(48)	(48)	(48)
Mineralization		1 (2%)		
Necrosis		2 (4%)		1 (2%)
Tongue	(1)			
Tooth	(15)	(8)	(8)	(6)
Malformation	14 (93%)	7 (88%)	8 (100%)	6 (100%)

^a Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	10 (20%)	18 (36%)	13 (26%)	17 (34%)
Inflammation, acute				2 (4%)
Mineralization		1 (2%)		1 (2%)
Necrosis				1 (2%)
Thrombosis	1 (2%)		1 (2%)	2 (4%)
Artery, inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Atrophy				1 (2%)
Degeneration		1 (2%)		
Hyperplasia	14 (28%)	15 (30%)	12 (24%)	8 (16%)
Hypertrophy	29 (58%)	16 (32%)	14 (29%)	9 (18%)
Adrenal medulla	(50)	(50)	(48)	(50)
Hyperplasia	2 (4%)	1 (2%)		
Islets, pancreatic	(50)	(50)	(49)	(49)
Hyperplasia	1 (2%)	3 (6%)	1 (2%)	
Hypertrophy			1 (2%)	
Pituitary gland	(48)	(49)	(49)	(49)
Pars distalis, hyperplasia	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Thyroid gland	(50)	(50)	(49)	(50)
Follicular cell, hyperplasia	7 (14%)	7 (14%)	7 (14%)	11 (22%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Granuloma sperm		2 (4%)		1 (2%)
Preputial gland	(49)	(50)	(50)	(50)
Ectasia		1 (2%)	1 (2%)	
Inflammation, chronic active	1 (2%)			
Prostate	(50)	(49)	(48)	(49)
Angiectasis	1 (2%)			
Hyperplasia		1 (2%)		1 (2%)
Infiltration cellular, polymorphonuclear				1 (2%)
Artery, inflammation, chronic active	1 (2%)			
Testes	(50)	(50)	(49)	(50)
Atrophy	5 (10%)	3 (6%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(49)
Thrombosis		1 (2%)		
Lymph node			(1)	(2)
Iliac, infiltration cellular, mixed cell			1 (100%)	
Lymph node, bronchial	(45)	(41)	(45)	(35)
Lymph node, mandibular	(34)	(35)	(22)	(25)
Infiltration cellular, plasma cell	1 (3%)			
Lymph node, mediastinal	(37)	(42)	(40)	(39)

TABLE C4

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Hematopoietic System (continued)				
Lymph node, mesenteric	(48)	(47)	(46)	(44)
Infiltration cellular, mixed cell			1 (2%)	
Infiltration cellular, plasma cell	1 (2%)		1 (2%)	
Spleen	(50)	(50)	(49)	(50)
Hematopoietic cell proliferation	3 (6%)	1 (2%)	2 (4%)	
Infiltration cellular, plasma cell	1 (2%)			
Thymus	(45)	(44)	(38)	(44)
Artery, inflammation			1 (3%)	
Integumentary System				
Skin	(50)	(50)	(49)	(50)
Cyst epithelial inclusion		1 (2%)		
Inflammation, acute				1 (2%)
Inflammation, chronic active	1 (2%)	2 (4%)	6 (12%)	3 (6%)
Ulcer				1 (2%)
Epidermis, abscess				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	1 (2%)			
Skeletal muscle			(1)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Degeneration		1 (2%)		
Gliosis				1 (2%)
Necrosis			1 (2%)	
Ventricle, infiltration cellular, polymorphonuclear				1 (2%)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Metaplasia, squamous				1 (2%)
Lung	(50)	(50)	(50)	(50)
Thrombosis		1 (2%)	1 (2%)	1 (2%)
Alveolar epithelium, bronchiole, metaplasia	5 (10%)	43 (86%)	42 (84%)	39 (78%)
Alveolar epithelium, hyperplasia	3 (6%)	3 (6%)	4 (8%)	
Alveolus, infiltration cellular, histiocyte	2 (4%)	3 (6%)		1 (2%)
Bronchiole, hyperplasia		11 (22%)	17 (34%)	18 (36%)
Bronchus, inflammation, acute			1 (2%)	
Bronchus, necrosis				1 (2%)
Nose	(50)	(50)	(49)	(48)
Inflammation, suppurative	2 (4%)	2 (4%)	9 (18%)	6 (13%)
Polyp, inflammatory	1 (2%)			
Glands, olfactory epithelium, hyperplasia	3 (6%)	11 (22%)	9 (18%)	23 (48%)
Olfactory epithelium, accumulation, hyaline droplet	1 (2%)			
Olfactory epithelium, atrophy	4 (8%)	13 (26%)	11 (22%)	38 (79%)
Olfactory epithelium, hyperplasia, atypical			5 (10%)	11 (23%)
Olfactory epithelium, hyperplasia, basal cell			15 (31%)	33 (69%)
Respiratory epithelium, accumulation, hyaline droplet	1 (2%)			
Respiratory epithelium, metaplasia, squamous	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Vomer nasal organ, inflammation, suppurative			1 (2%)	

TABLE C4

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Respiratory System (continued)				
Pleura				(2)
Trachea	(50)	(50)	(49)	(50)
Necrosis				1 (2%)
Special Senses System				
Eye	(49)	(50)	(49)	(48)
Cataract	1 (2%)	1 (2%)		1 (2%)
Cornea, inflammation, chronic active		2 (4%)	1 (2%)	4 (8%)
Cornea, mineralization				1 (2%)
Harderian gland	(48)	(50)	(49)	(50)
Hyperplasia	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Hypertrophy				1 (2%)
Inflammation, chronic	1 (2%)			
Necrosis				1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct	1 (2%)	4 (8%)	3 (6%)	7 (14%)
Inflammation, suppurative				1 (2%)
Metaplasia, osseous	3 (6%)	1 (2%)		1 (2%)
Mineralization	1 (2%)	1 (2%)		2 (4%)
Nephropathy	47 (94%)	44 (88%)	45 (90%)	41 (82%)
Artery, inflammation, chronic active	1 (2%)			1 (2%)
Capsule, fibrosis		1 (2%)		
Perirenal tissue, thrombosis				1 (2%)
Renal tubule, cyst	1 (2%)		1 (2%)	
Renal tubule, hyperplasia		2 (4%)		
Renal tubule, necrosis				1 (2%)
Renal tubule, pigmentation				1 (2%)
Urinary bladder	(50)	(48)	(49)	(48)

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

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TABLE D1

Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	125 ppm	250 ppm	500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	8	10	8	12
Natural deaths	5	4	3	3
Survivors				
Died last week of study				1
Terminal sacrifice	37	36	39	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Squamous cell carcinoma				1 (2%)
Gallbladder	(38)	(41)	(44)	(37)
Intestine large, cecum	(46)	(47)	(49)	(47)
Intestine large, colon	(47)	(50)	(50)	(48)
Intestine small, duodenum	(46)	(47)	(49)	(48)
Carcinoma		1 (2%)		
Intestine small, ileum	(46)	(47)	(49)	(47)
Intestine small, jejunum	(46)	(47)	(49)	(47)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Hepatoblastoma			1 (2%)	
Hepatocellular adenoma	9 (18%)	10 (20%)	18 (36%)	19 (38%)
Hepatocellular adenoma, multiple	9 (18%)	13 (26%)	9 (18%)	10 (20%)
Hepatocellular carcinoma	8 (16%)	6 (12%)	4 (8%)	12 (24%)
Hepatocellular carcinoma, multiple	2 (4%)	1 (2%)	2 (4%)	
Hepatocholangiocarcinoma		1 (2%)		
Ito cell tumor benign				1 (2%)
Mesentery	(11)	(12)	(11)	(9)
Hemangiosarcoma			1 (9%)	
Sarcoma		1 (8%)		
Pancreas	(49)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)	2 (4%)	2 (4%)
Epithelium, squamous cell carcinoma	1 (2%)			
Stomach, glandular	(49)	(50)	(50)	(49)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, uncertain primary site	1 (2%)			1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Adrenal medulla	(50)	(49)	(50)	(49)
Pheochromocytoma malignant			2 (4%)	1 (2%)

TABLE D1

Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Endocrine System (continued)				
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma		1 (2%)	1 (2%)	1 (2%)
Pituitary gland	(50)	(49)	(50)	(48)
Pars distalis, adenoma	8 (16%)	6 (12%)	6 (12%)	10 (21%)
Pars intermedia, adenoma	2 (4%)		1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Bilateral, follicular cell, carcinoma		1 (2%)		
C-cell, adenoma		1 (2%)		
Follicular cell, adenoma	1 (2%)	4 (8%)		3 (6%)
Follicular cell, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Ovary	(48)	(50)	(50)	(49)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Cystadenocarcinoma		1 (2%)	1 (2%)	
Cystadenoma	5 (10%)	2 (4%)		3 (6%)
Luteoma			1 (2%)	
Teratoma benign	1 (2%)			
Yolk sac carcinoma		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Leiomyoma			1 (2%)	
Polyp stromal	3 (6%)		1 (2%)	2 (4%)
Bilateral, polyp stromal			1 (2%)	
Endometrium, carcinoma	3 (6%)		1 (2%)	
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hemangiosarcoma			2 (4%)	
Lymph node	(4)	(9)	(5)	(5)
Axillary, hemangiosarcoma, metastatic, spleen			1 (20%)	
Renal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (20%)	
Renal, hepatocholangiocarcinoma, metastatic, liver		1 (11%)		
Lymph node, bronchial	(39)	(36)	(40)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, uncertain primary site	1 (3%)			
Lymph node, mandibular	(35)	(42)	(38)	(42)
Lymph node, mediastinal	(44)	(40)	(42)	(40)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	2 (5%)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver		1 (3%)		
Lymph node, mesenteric	(46)	(46)	(48)	(48)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Spleen	(49)	(50)	(50)	(50)
Hemangiosarcoma			3 (6%)	1 (2%)

TABLE D1

Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Hematopoietic System (continued)				
Thymus	(49)	(50)	(48)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	1 (2%)		
Fibroadenoma				1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma			1 (2%)	
Squamous cell carcinoma	1 (2%)			
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, hemangioma				1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	2 (4%)	1 (2%)	
Subcutaneous tissue, sarcoma		1 (2%)	1 (2%)	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Skeletal muscle	(1)	(1)	(1)	
Carcinoma, metastatic, uncertain primary site	1 (100%)			
Hemangiosarcoma			1 (100%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Squamous cell papilloma				1 (2%)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	13 (26%)	16 (32%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple		13 (26%)	20 (40%)	30 (60%)
Alveolar/bronchiolar carcinoma	3 (6%)	10 (20%)	13 (26%)	15 (30%)
Alveolar/bronchiolar carcinoma, multiple		6 (12%)	7 (14%)	19 (38%)
Carcinoma, metastatic, Harderian gland	3 (6%)			
Carcinoma, metastatic, uncertain primary site	1 (2%)			1 (2%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	5 (10%)	2 (4%)	3 (6%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland	1 (2%)			
Hemangioma				1 (2%)
Hemangiosarcoma			1 (2%)	
Respiratory epithelium, adenoma		1 (2%)		

TABLE D1

Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Respiratory System (continued)				
Pleura		(1)	(1)	(2)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)	1 (50%)
Carcinoma, metastatic, uncertain primary site				1 (50%)
Hepatocholangiocarcinoma, metastatic, liver		1 (100%)		
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(50)	(50)	(49)
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	8 (16%)	4 (8%)		2 (4%)
Carcinoma	5 (10%)	1 (2%)	1 (2%)	5 (10%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, uncertain primary site	1 (2%)			1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Urinary bladder	(48)	(48)	(49)	(48)
Transitional epithelium, carcinoma			1 (2%)	
Systemic Lesions				
Multiple organs ^b	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	2 (4%)	
Lymphoma malignant	7 (14%)	15 (30%)	6 (12%)	12 (24%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	42	44	50	50
Total primary neoplasms	81	120	131	163
Total animals with benign neoplasms	34	39	47	47
Total benign neoplasms	48	68	76	94
Total animals with malignant neoplasms	26	33	37	43
Total malignant neoplasms	33	52	55	69
Total animals with metastatic neoplasms	7	6	3	7
Total metastatic neoplasms	16	14	6	19
Total animals with malignant neoplasms-uncertain primary site	1			1

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2

Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Harderian Gland: Adenoma				
Overall rate ^a	8/50 (16%)	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted rate ^b	18.0%	8.9%	0.0%	4.3%
Terminal rate ^c	7/37 (19%)	3/36 (8%)	0/39 (0%)	2/35 (6%)
First incidence (days) ^d	656	726	— ^e	731 (T)
Poly-3 test	P=0.012N	P=0.171N	P=0.003N	P=0.036N
Harderian Gland: Carcinoma				
Overall rate	5/50 (10%)	1/50 (2%)	1/50 (2%)	5/50 (10%)
Adjusted rate	11.2%	2.2%	2.1%	10.6%
Terminal rate	3/37 (8%)	1/36 (3%)	1/39 (3%)	3/35 (9%)
First incidence (days)	683	731 (T)	731 (T)	672
Poly-3 test	P=0.441	P=0.099N	P=0.090N	P=0.591N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	12/50 (24%)	5/50 (10%)	1/50 (2%)	6/50 (12%)
Adjusted rate	26.8%	11.2%	2.1%	12.7%
Terminal rate	9/37 (24%)	4/36 (11%)	1/39 (3%)	4/35 (11%)
First incidence (days)	656	726	731 (T)	672
Poly-3 test	P=0.056N	P=0.050N	P<0.001N	P=0.073N
Liver: Hepatocellular Adenoma				
Overall rate	18/50 (36%)	23/50 (46%)	27/50 (54%) ^f	29/50 (58%)
Adjusted rate	40.5%	50.0%	56.4%	59.8%
Terminal rate	17/37 (46%)	17/36 (47%)	22/39 (56%)	19/35 (54%)
First incidence (days)	654	609	618	662
Poly-3 test	P=0.040	P=0.243	P=0.091	P=0.046
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	7/50 (14%)	6/50 (12%)	12/50 (24%)
Adjusted rate	22.2%	15.5%	12.7%	25.4%
Terminal rate	7/37 (19%)	5/36 (14%)	4/39 (10%)	10/35 (29%)
First incidence (days)	607	623	651	702
Poly-3 test	P=0.311	P=0.291N	P=0.177N	P=0.455
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/50 (50%)	26/50 (52%)	29/50 (58%) ^f	36/50 (72%)
Adjusted rate	55.6%	56.5%	60.4%	74.1%
Terminal rate	22/37 (60%)	20/36 (56%)	23/39 (59%)	25/35 (71%)
First incidence (days)	607	609	618	662
Poly-3 test	P=0.024	P=0.549	P=0.395	P=0.043
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/50 (20%)	7/50 (14%)	7/50 (14%)	12/50 (24%)
Adjusted rate	22.2%	15.5%	14.7%	25.4%
Terminal rate	7/37 (19%)	5/36 (14%)	4/39 (10%)	10/35 (29%)
First incidence (days)	607	623	618	702
Poly-3 test	P=0.305	P=0.291N	P=0.255N	P=0.455
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	26/50 (52%)	36/50 (72%)	38/50 (76%)
Adjusted rate	2.3%	56.3%	74.5%	77.9%
Terminal rate	1/37 (3%)	21/36 (58%)	31/39 (80%)	29/35 (83%)
First incidence (days)	731 (T)	555	495	565
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

TABLE D2

Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	16/50 (32%)	20/50 (40%)	34/50 (68%)
Adjusted rate	6.7%	35.3%	41.9%	69.5%
Terminal rate	2/37 (5%)	13/36 (36%)	15/39 (39%)	24/35 (69%)
First incidence (days)	533	646	618	513
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	31/50 (62%)	42/50 (84%)	46/50 (92%)
Adjusted rate	8.9%	66.8%	86.0%	92.4%
Terminal rate	3/37 (8%)	25/36 (69%)	34/39 (87%)	33/35 (94%)
First incidence (days)	533	555	495	513
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Ovary: Cystadenoma				
Overall rate	5/48 (10%)	2/50 (4%)	0/50 (0%)	3/49 (6%)
Adjusted rate	11.5%	4.5%	0.0%	6.5%
Terminal rate	5/37 (14%)	2/36 (6%)	0/39 (0%)	3/34 (9%)
First incidence (days)	731 (T)	731 (T)	—	731 (T)
Poly-3 test	P=0.274N	P=0.204N	P=0.025N	P=0.326N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	8/50 (16%)	6/49 (12%)	6/50 (12%)	10/48 (21%)
Adjusted rate	18.1%	13.6%	12.8%	21.8%
Terminal rate	8/37 (22%)	6/36 (17%)	5/39 (13%)	8/34 (24%)
First incidence (days)	731 (T)	731 (T)	709	565
Poly-3 test	P=0.299	P=0.385N	P=0.342N	P=0.433
Spleen: Hemangiosarcoma				
Overall rate	0/49 (0%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	6.4%	2.1%
Terminal rate	0/37 (0%)	0/36 (0%)	2/39 (5%)	1/35 (3%)
First incidence (days)	—	— ^g	673	731 (T)
Poly-3 test	P=0.271	— ^g	P=0.130	P=0.513
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/50 (2%)	4/50 (8%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	8.9%	0.0%	6.4%
Terminal rate	1/37 (3%)	4/36 (11%)	0/39 (0%)	3/35 (9%)
First incidence (days)	731 (T)	731 (T)	—	731 (T)
Poly-3 test	P=0.432	P=0.183	P=0.489N	P=0.329
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	0/50 (0%)	3/50 (6%)
Adjusted rate	4.5%	11.2%	0.0%	6.4%
Terminal rate	1/37 (3%)	5/36 (14%)	0/39 (0%)	3/35 (9%)
First incidence (days)	683	731 (T)	—	731 (T)
Poly-3 test	P=0.519N	P=0.220	P=0.226N	P=0.527

TABLE D2

Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Uterus: Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.8%	0.0%	2.1%	0.0%
Terminal rate	3/37 (8%)	0/36 (0%)	1/39 (3%)	0/35 (0%)
First incidence (days)	731 (T)	—	731 (T)	—
Poly-3 test	P=0.081N	P=0.116N	P=0.285N	P=0.108N
Uterus: Stromal Polyp				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	2/50 (4%)
Adjusted rate	6.8%	0.0%	4.3%	4.2%
Terminal rate	2/37 (5%)	0/36 (0%)	2/39 (5%)	1/35 (3%)
First incidence (days)	693	—	731 (T)	677
Poly-3 test	P=0.560N	P=0.116N	P=0.476N	P=0.471N
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	6/50 (12%)	1/50 (2%)
Adjusted rate	2.3%	6.6%	12.8%	2.1%
Terminal rate	1/37 (3%)	1/36 (3%)	5/39 (13%)	1/35 (3%)
First incidence (days)	731 (T)	555	673	731 (T)
Poly-3 test	P=0.518N	P=0.318	P=0.066	P=0.746N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	6/50 (12%)	3/50 (6%)
Adjusted rate	2.3%	6.6%	12.8%	6.4%
Terminal rate	1/37 (3%)	1/36 (3%)	5/39 (13%)	3/35 (9%)
First incidence (days)	731 (T)	555	673	731 (T)
Poly-3 test	P=0.304	P=0.318	P=0.066	P=0.329
All Organs: Malignant Lymphoma				
Overall rate	7/50 (14%)	15/50 (30%)	6/50 (12%)	12/50 (24%)
Adjusted rate	15.4%	33.0%	12.7%	25.0%
Terminal rate	5/37 (14%)	12/36 (33%)	4/39 (10%)	7/35 (20%)
First incidence (days)	292	645	639	565
Poly-3 test	P=0.372	P=0.041	P=0.471N	P=0.187
All Organs: Benign Neoplasms				
Overall rate	34/50 (68%)	39/50 (78%)	47/50 (94%)	47/50 (94%)
Adjusted rate	73.3%	82.6%	95.7%	95.3%
Terminal rate	29/37 (78%)	30/36 (83%)	38/39 (97%)	34/35 (97%)
First incidence (days)	193	555	495	565
Poly-3 test	P<0.001	P=0.192	P<0.001	P=0.002
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	33/50 (66%)	37/50 (74%)	43/50 (86%)
Adjusted rate	55.3%	67.6%	74.0%	86.0%
Terminal rate	19/37 (51%)	21/36 (58%)	26/39 (67%)	28/35 (80%)
First incidence (days)	292	481	495	513
Poly-3 test	P<0.001	P=0.149	P=0.040	P<0.001

TABLE D2

Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	42/50 (84%)	44/50 (88%)	50/50 (100%)	50/50 (100%)
Adjusted rate	85.9%	90.2%	100%	100%
Terminal rate	32/37 (87%)	32/36 (89%)	39/39 (100%)	35/35 (100%)
First incidence (days)	193	481	495	513
Poly-3 test	P<0.001	P=0.366	P=0.007	P=0.007

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, pituitary gland, spleen, and thyroid gland; for other tissues, denominator is number of animals necropsied.

^b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by N.

^e Not applicable; no neoplasms in animal group

^f One animal with adenoma also had hepatoblastoma.

^g Value of statistic cannot be computed.

TABLE D3a

Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1 Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
Cumene	1/50	3/50	4/50
Decalin	1/49	6/49	7/49
Divinylbenzene	4/50	2/50	6/50
Methyl isobutyl ketone	4/50	0/50	4/50
α -Methylstyrene	1/50	1/50	2/50
Propargyl alcohol	3/50	2/50	5/50
Propylene glycol mono- <i>t</i> -butyl ether	2/50	1/50	3/50
Stoddard solvent IIC	2/50	0/50	2/50
Vanadium pentoxide	1/50	0/50	1/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	19/449 (4.2%)	15/449 (3.3%)	34/449 (7.6%)
Mean \pm standard deviation	4.2% \pm 2.5%	3.4% \pm 3.9%	7.6% \pm 4.0%
Range	2%-8%	0%-12%	2%-14%
Overall Historical Incidence: All Routes			
Total (%)	77/1,596 (4.8%)	57/1,596 (3.6%)	129/1,596 (8.1%)
Mean \pm standard deviation	4.9% \pm 2.7%	3.6% \pm 3.1%	8.2% \pm 3.9%
Range	0%-12%	0%-12%	2%-18%

^a Data as of March 2, 2007

TABLE D3b
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F1 Mice^a

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
Cumene	18/50	10/50	25/50
Decalin	7/49	4/49	11/49
Divinylbenzene	17/49	5/49	19/49
Methyl isobutyl ketone	13/50	6/50	17/50
α -Methylstyrene	10/50	3/50	13/50
Propargyl alcohol	15/50	4/50	17/50
Propylene glycol mono- <i>t</i> -butyl ether	14/49	4/49	18/49
Stoddard solvent IIC	9/50	6/50	13/50
Vanadium pentoxide	6/50	6/50	12/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	109/447 (24.4%)	48/447 (10.7%)	145/447 (32.4%)
Mean \pm standard deviation	24.4% \pm 8.7%	10.7% \pm 4.1%	32.4% \pm 8.8%
Range	12%-36%	6%-20%	22%-50%
Overall Historical Incidence: All Routes			
Total (%)	402/1,593 (25.2%)	159/1,593 (10.0%)	505/1,593 (31.7%)
Mean \pm standard deviation	25.8% \pm 15.8%	10.2% \pm 6.6%	32.4% \pm 17.5%
Range	2%-62%	0%-28%	8%-64%

^a Data as of March 2, 2007

TABLE D3c
Historical Incidence of Hemangiosarcoma in Control Female B6C3F1 Mice^a

Study	Incidence in Controls	
	Spleen	All Organs
Historical Incidence: Inhalation Studies		
Cumene	0/49	1/50
Decalin	1/49	1/49
Divinylbenzene	0/49	1/50
Methyl isobutyl ketone	1/50	3/50
α -Methylstyrene	0/50	4/50
Propargyl alcohol	1/50	2/50
Propylene glycol mono- <i>t</i> -butyl ether	0/49	1/50
Stoddard solvent IIC	1/49	1/50
Vanadium pentoxide	2/50	2/50
Overall Historical Incidence: Inhalation Studies		
Total (%)	6/445 (1.4%)	16/449 (3.6%)
Mean \pm standard deviation	1.3% \pm 1.4%	3.6% \pm 2.2%
Range	0%-4%	2%-8%
Overall Historical Incidence: All Routes		
Total (%)	27/1,573 (1.7%)	71/1,598 (4.4%)
Mean \pm standard deviation	1.8% \pm 1.8%	4.6% \pm 3.2%
Range	0%-8%	2%-16%

^a Data as of March 2, 2007

TABLE D4

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene^a

	Chamber Control	125 ppm	250 ppm	500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	8	10	8	12
Natural deaths	5	4	3	3
Survivors				
Died last week of study				1
Terminal sacrifice	37	36	39	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(38)	(41)	(44)	(37)
Intestine large, cecum	(46)	(47)	(49)	(47)
Intestine large, colon	(47)	(50)	(50)	(48)
Intestine small, duodenum	(46)	(47)	(49)	(48)
Necrosis	1 (2%)			
Intestine small, ileum	(46)	(47)	(49)	(47)
Intestine small, jejunum	(46)	(47)	(49)	(47)
Hyperplasia, lymphoid			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	1 (2%)	
Basophilic focus	3 (6%)	3 (6%)	4 (8%)	1 (2%)
Clear cell focus	4 (8%)	6 (12%)	1 (2%)	5 (10%)
Cyst	1 (2%)			1 (2%)
Eosinophilic focus	8 (16%)	11 (22%)	7 (14%)	14 (28%)
Erythrophagocytosis				1 (2%)
Fatty change	1 (2%)			
Hematopoietic cell proliferation		1 (2%)		
Inflammation, granulomatous	1 (2%)			
Mixed cell focus		1 (2%)		1 (2%)
Necrosis	4 (8%)	3 (6%)	3 (6%)	2 (4%)
Tension lipidosis	3 (6%)	3 (6%)	4 (8%)	4 (8%)
Centrilobular, necrosis	2 (4%)	1 (2%)		1 (2%)
Mesentery	(11)	(12)	(11)	(9)
Inflammation, chronic active			1 (9%)	
Fat, hemorrhage		1 (8%)		
Fat, necrosis	11 (100%)	10 (83%)	10 (91%)	9 (100%)
Pancreas	(49)	(50)	(50)	(50)
Atrophy			1 (2%)	3 (6%)
Inflammation, chronic active	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation	1 (2%)			
Ulcer			1 (2%)	2 (4%)
Epithelium, erosion				1 (2%)
Epithelium, hyperplasia	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Stomach, glandular	(49)	(50)	(50)	(49)
Mineralization			1 (2%)	
Necrosis	1 (2%)			1 (2%)

^a Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D4

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	12 (24%)	17 (34%)	6 (12%)	7 (14%)
Inflammation, chronic			1 (2%)	
Mineralization		1 (2%)	1 (2%)	
Thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Hyperplasia	5 (10%)	7 (14%)	7 (14%)	5 (10%)
Hypertrophy	2 (4%)	1 (2%)	1 (2%)	6 (12%)
Adrenal medulla	(50)	(49)	(50)	(49)
Hyperplasia	1 (2%)	4 (8%)	2 (4%)	2 (4%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Pituitary gland	(50)	(49)	(50)	(48)
Pars distalis, angiectasis	3 (6%)	1 (2%)	7 (14%)	1 (2%)
Pars distalis, hyperplasia	11 (22%)	19 (39%)	10 (20%)	6 (13%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, hyperplasia	15 (30%)	10 (20%)	16 (32%)	10 (20%)
General Body System				
None				
Genital System				
Ovary	(48)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Cyst	9 (19%)	12 (24%)	14 (28%)	11 (22%)
Thrombosis	1 (2%)	1 (2%)		1 (2%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Decidual reaction	1 (2%)			
Inflammation, suppurative	1 (2%)	2 (4%)		1 (2%)
Thrombosis	1 (2%)			2 (4%)
Endometrium, hyperplasia, cystic	8 (16%)	11 (22%)	18 (36%)	8 (16%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Lymph node	(4)	(9)	(5)	(5)
Iliac, ectasia	1 (25%)	1 (11%)		
Lumbar, angiectasis		1 (11%)		
Lumbar, ectasia	2 (50%)	1 (11%)	1 (20%)	1 (20%)
Lumbar, hyperplasia, lymphoid		2 (22%)		
Renal, ectasia	1 (25%)			
Lymph node, bronchial	(39)	(36)	(40)	(46)
Lymph node, mandibular	(35)	(42)	(38)	(42)
Inflammation, granulomatous	1 (3%)			
Lymph node, mediastinal	(44)	(40)	(42)	(40)
Hyperplasia, lymphoid				1 (3%)
Lymph node, mesenteric	(46)	(46)	(48)	(48)

TABLE D4

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Hematopoietic System (continued)				
Spleen	(49)	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)	6 (12%)	2 (4%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Thymus	(49)	(50)	(48)	(48)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			1 (2%)
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(1)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Necrosis			1 (2%)	
Meninges, infiltration cellular, mononuclear cell				1 (2%)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Inflammation, suppurative				1 (2%)
Metaplasia, squamous	1 (2%)			
Lung	(50)	(50)	(50)	(50)
Foreign body				1 (2%)
Hemorrhage		2 (4%)		
Inflammation, suppurative				1 (2%)
Thrombosis		2 (4%)		
Alveolar epithelium, bronchiole, hyperplasia			1 (2%)	
Alveolar epithelium, bronchiole, metaplasia		42 (84%)	49 (98%)	47 (94%)
Alveolar epithelium, hyperplasia	2 (4%)	3 (6%)	6 (12%)	6 (12%)
Alveolar epithelium, metaplasia, squamous	1 (2%)			
Alveolus, infiltration cellular, histiocyte		1 (2%)	1 (2%)	3 (6%)
Bronchiole, hyperplasia		17 (34%)	10 (20%)	14 (28%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	3 (6%)	7 (14%)
Glands, olfactory epithelium, cyst			1 (2%)	
Glands, olfactory epithelium, hyperplasia	1 (2%)	4 (8%)	4 (8%)	11 (22%)
Olfactory epithelium, atrophy	4 (8%)	11 (22%)	9 (18%)	18 (36%)
Olfactory epithelium, hyperplasia, atypical			2 (4%)	10 (20%)
Olfactory epithelium, hyperplasia, basal cell		1 (2%)	11 (22%)	25 (50%)
Olfactory epithelium, necrosis		2 (4%)	2 (4%)	3 (6%)
Respiratory epithelium, accumulation, hyaline droplet		1 (2%)	1 (2%)	
Respiratory epithelium, metaplasia, squamous			1 (2%)	6 (12%)
Respiratory epithelium, necrosis			1 (2%)	
Turbinates, necrosis				2 (4%)
Pleura		(1)	(1)	(2)
Trachea	(50)	(50)	(50)	(50)
Inflammation, suppurative				2 (4%)

TABLE D4

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene

	Chamber Control	125 ppm	250 ppm	500 ppm
Special Senses System				
Eye	(49)	(50)	(50)	(49)
Cataract	2 (4%)		1 (2%)	2 (4%)
Degeneration				1 (2%)
Cornea, epithelium, hyperplasia				1 (2%)
Cornea, inflammation, acute	1 (2%)		1 (2%)	
Cornea, inflammation, chronic active	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Cornea, mineralization				1 (2%)
Harderian gland	(50)	(49)	(50)	(50)
Atrophy	1 (2%)			
Hyperplasia	6 (12%)	2 (4%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst		1 (2%)		
Infarct	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Inflammation, suppurative				1 (2%)
Metaplasia, osseous	2 (4%)	3 (6%)	1 (2%)	
Nephropathy	36 (73%)	34 (68%)	34 (68%)	34 (68%)
Capsule, fibrosis	1 (2%)		1 (2%)	
Renal tubule, karyomegaly	1 (2%)			
Renal tubule, necrosis	3 (6%)	1 (2%)		1 (2%)
Urinary bladder	(48)	(48)	(49)	(48)
Inflammation, chronic active			1 (2%)	
Artery, inflammation, chronic active		1 (2%)		

APPENDIX E

GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

***SALMONELLA TYPHIMURIUM* MUTAGENICITY TEST PROTOCOL**

Testing was performed as reported by Zeiger *et al.* (1988). Cumene was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of cumene. The high dose was limited by toxicity. All trials were repeated; negative trials conducted with S9 were repeated with a higher S9 concentration.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

RAT BONE MARROW MICRONUCLEUS TEST PROTOCOL

Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by cumene exposure. A high dose of 2,500 mg/kg was selected based on toxicity. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats were injected intraperitoneally (three times at 24-hour intervals) with cumene dissolved in corn oil. Vehicle control animals were injected with corn oil only. The positive control animals received injections of cyclophosphamide (25 mg/kg). The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in up to five rats per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of cumene-induced bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted previously). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female B6C3F1 mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronucleated cells in 1,000 normochromatic erythrocytes (NCEs) in each of 9 or 10 mice per exposure group. In addition, the percentage of PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as described for PCEs in the rat bone marrow micronucleus test. Results of the 3-month study were accepted without repeat tests because additional data could not be obtained.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

Cumene (1 to 333 µg/plate) was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535 when tested with and without induced rat or hamster liver S9 activation enzymes (Table E1). *In vivo*, cumene induced small, but significant, increases in micronucleated PCEs in bone marrow of male rats treated by intraperitoneal injection (Table E2). Two trials were performed in rats. In the first trial, doses ranging from 78.13 to 2,500 mg/kg were administered three times at 24-hour intervals, and results were positive, based both on the trend ($P < 0.001$) and the response at the 1,250 mg/kg dose. The data from the 2,500 mg/kg dose were excluded from analysis because only two animals survived and a minimum of three animals is required for a valid dose point. The second confirmatory trial also produced a positive response, although the trend test was not significant ($P = 0.085$). Micronucleated erythrocytes were elevated at all four doses in trial 2; the responses at the 312 and 1,250 mg/kg levels were statistically significant ($P < 0.006$). Because considerable toxicity was again observed at the high dose of 2,500 mg/kg, the trend was recalculated over the same dose range as for the first trial (0 to 1,250 mg/kg), and a significant P value of 0.019 was produced. The percentage of PCEs in the bone marrow fluctuated unrelated to dose and likely represented variation within the normal range of 40% to 60% PCEs among the total erythrocyte population in the bone marrow. In contrast to the results in male rats, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation (62.5 to 1,000 ppm) for 3 months (Table E3). For both male and female mice, no significant changes in the percentage of PCEs were observed over the exposure range tested, indicating an absence of treatment-related toxicity to the bone marrow.

TABLE E1
Mutagenicity of Cumene in *Salmonella typhimurium*^a

Strain	Dose (µg/plate)	Revertants/Plate ^b					
		-S9		+hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
TA100	0	99 ± 5.0	105 ± 1.0	121 ± 11.0	126 ± 20.0	125 ± 7.0	125 ± 11.0
	1		100 ± 8.0				
	3	103 ± 3.0	104 ± 4.0	107 ± 4.0	119 ± 6.0	112 ± 8.0	137 ± 11.0
	10	93 ± 6.0	116 ± 7.0	112 ± 8.0	127 ± 8.0	116 ± 10.0	129 ± 3.0
	33	96 ± 5.0	93 ± 6.0	102 ± 7.0	105 ± 7.0	113 ± 5.0	122 ± 4.0
	100	100 ± 1.0	111 ± 8.0	102 ± 3.0	112 ± 7.0	105 ± 3.0	138 ± 5.0
	166	47 ± 14.0 ^c		106 ± 6.0		95 ± 5.0	
	333				79 ± 4.0 ^c		91 ± 9.0
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control ^d	914 ± 8.0	875 ± 26.0	1,420 ± 121.0	603 ± 25.0	1,111 ± 30.0	792 ± 16.0
TA1535	0	15 ± 1.0	14 ± 0.0	15 ± 2.0	14 ± 1.0	21 ± 2.0	13 ± 3.0
	1	10 ± 2.0	13 ± 2.0				
	3	17 ± 0.0	13 ± 1.0	12 ± 2.0	12 ± 1.0	17 ± 2.0	14 ± 0.0
	10	14 ± 1.0	13 ± 3.0	17 ± 1.0	9 ± 2.0	17 ± 1.0	14 ± 2.0
	33	13 ± 1.0	12 ± 1.0	11 ± 1.0	14 ± 3.0	15 ± 2.0	12 ± 2.0
	100	11 ± 2.0	16 ± 5.0	10 ± 2.0	15 ± 1.0	12 ± 2.0	12 ± 2.0
	166			9 ± 1.0	10 ± 2.0	11 ± 1.0	11 ± 4.0
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control	851 ± 8.0	399 ± 17.0	198 ± 12.0	321 ± 15.0	140 ± 2.0	222 ± 7.0
TA97	0	133 ± 12.0	156 ± 7.0	188 ± 2.0	159 ± 1.0	192 ± 4.0	170 ± 11.0
	1	128 ± 1.0	177 ± 3.0				
	3	144 ± 5.0	174 ± 2.0	181 ± 6.0	165 ± 6.0	172 ± 8.0	189 ± 5.0
	10	148 ± 12.0	172 ± 2.0	185 ± 3.0	171 ± 4.0	176 ± 7.0	191 ± 3.0
	33	136 ± 13.0	158 ± 4.0	195 ± 9.0	185 ± 4.0	190 ± 4.0	188 ± 2.0
	100	99 ± 8.0	53 ± 17.0 ^c	153 ± 1.0	186 ± 7.0	149 ± 15.0	168 ± 15.0
	166			125 ± 9.0 ^c	153 ± 4.0	115 ± 9.0 ^c	163 ± 2.0
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control	426 ± 13.0	515 ± 13.0	545 ± 22.0	481 ± 18.0	478 ± 12.0	435 ± 23.0
TA98	0	9 ± 1.0	20 ± 1.0	22 ± 3.0	14 ± 3.0	19 ± 1.0	19 ± 1.0
	1		22 ± 4.0				
	3	9 ± 0.0	16 ± 3.0	22 ± 2.0	14 ± 2.0	25 ± 5.0	11 ± 2.0
	10	9 ± 1.0	20 ± 2.0	23 ± 1.0	13 ± 3.0	24 ± 3.0	14 ± 3.0
	33	14 ± 1.0	18 ± 5.0	22 ± 3.0	13 ± 3.0	16 ± 1.0	13 ± 1.0
	100	8 ± 0.0 ^c	16 ± 2.0	23 ± 2.0	18 ± 2.0	14 ± 1.0	11 ± 2.0
	166	4 ± 0.0 ^c		21 ± 3.0		23 ± 1.0	
	333				10 ± 1.0 ^c		12 ± 3.0
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control	328 ± 28.0	414 ± 24.0	1,227 ± 35.0	291 ± 13.0	890 ± 63.0	669 ± 17.0

^a Study performed at SRI International. The detailed protocol is presented by Zeiger *et al.* (1988). 0 µg/plate was the solvent control.

^b Revertants are presented as mean ± standard error from three plates.

^c Slight toxicity

^d The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Cumene by Intraperitoneal Injection^a

Compound	Dose (mg/kg)	Number of Male Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs ^b	Pairwise P Value ^c	PCE ^b (%)
Trial 1					
Corn oil ^d	0	5	0.50 ± 0.16		50.2 ± 2.9
Cumene	78.13	5	1.20 ± 0.25	0.0447	59.4 ± 5.1
	156.25	5	1.20 ± 0.34	0.0447	64.8 ± 4.2
	312.5	5	1.30 ± 0.54	0.0296	54.6 ± 3.1
	625	5	0.80 ± 0.41	0.2026	45.1 ± 1.7
	1,250	5	2.60 ± 0.29	0.0001	46.6 ± 4.8
	2,500	2 ^e	1.25 ± 0.25		49.3 ± 2.8
			P<0.001 ^f		
Cyclophosphamide ^g	25	5	17.30 ± 2.32	0.0000	50.3 ± 4.3
Trial 2					
Corn oil	0	5	0.50 ± 0.27		53.2 ± 3.8
Cumene	312	5	1.70 ± 0.20	0.0052	50.2 ± 1.0
	625	5	1.40 ± 0.33	0.0194	47.6 ± 3.1
	1,250	5	1.80 ± 0.34	0.0033	44.5 ± 3.0
	2,500	3	1.50 ± 1.00	0.0192	54.3 ± 2.1
			P=0.085		
Cyclophosphamide	25	5	7.80 ± 1.63	0.0000	38.7 ± 2.7

^a Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al.* (1993).

^b PCE=polychromatic erythrocyte

^c Mean ± standard error

^d Pairwise comparison with the vehicle control; dosed group values are significant at P≤0.005 (trial 1) or P≤0.006 (trial 2); positive control values are significant at P≤0.05 (ILS, 1990)

^e Vehicle control

^f Statistical tests not performed due to high mortality

^g Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990); 2,500 mg/kg group excluded due to high mortality

^h Positive control

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice
Following Treatment with Cumene by Inhalation for 3 Months^a

Compound	Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs ^b	P-Value ^c	PCEs ^b (%)
Male					
Air ^d	0	10	2.40 ± 0.69		2.7 ± 0.1
Cumene	62.5	10	2.20 ± 0.66	0.6161	2.6 ± 0.1
	125	10	2.10 ± 0.48	0.6728	2.6 ± 0.1
	250	10	1.80 ± 0.36	0.8230	2.8 ± 0.1
	500	10	2.00 ± 0.26	0.7270	2.9 ± 0.1
	1,000	10	2.20 ± 0.42	0.6161	2.9 ± 0.2
			P=0.553 ^e		
Female					
Air	0	10	2.30 ± 0.40		3.3 ± 0.1
Cumene	62.5	9	1.33 ± 0.37	0.9396	2.3 ± 0.1
	125	10	1.70 ± 0.30	0.8289	3.1 ± 0.2
	250	10	2.10 ± 0.53	0.6186	3.3 ± 0.2
	500	10	2.10 ± 0.35	0.6186	3.4 ± 0.1
			P=0.329		

^a Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990).

^b NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte

^c Mean ± standard error

^d Pairwise comparison with the chamber controls, significant at P≤0.005 (males) or P≤0.006 (females) (ILS, 1990)

^e Chamber control

^e Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX F

CLINICAL PATHOLOGY RESULTS

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TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Male						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	9
Hematocrit (%)						
Day 3	43.6 ± 0.7	42.1 ± 0.4	42.7 ± 0.4	42.9 ± 0.5	42.9 ± 0.5	42.5 ± 0.4
Day 23	49.0 ± 0.5	48.4 ± 0.4	48.1 ± 0.5	47.7 ± 0.4	48.4 ± 0.3	48.9 ± 0.5
Week 14	45.5 ± 0.4	45.4 ± 0.3	45.1 ± 0.4	44.3 ± 0.3	44.3 ± 0.3	44.4 ± 0.2
Packed cell volume (mL/dL)						
Day 3	41.3 ± 0.6	40.1 ± 0.5	40.5 ± 0.5	40.8 ± 0.5	40.7 ± 0.5	39.9 ± 0.4
Day 23	48.0 ± 0.7	47.9 ± 0.5	47.2 ± 0.5	46.8 ± 0.5	46.9 ± 0.4	48.8 ± 0.5
Week 14	45.5 ± 0.3	45.4 ± 0.5	44.8 ± 0.4	44.4 ± 0.5	44.1 ± 0.4	44.7 ± 0.3
Hemoglobin (g/dL)						
Day 3	13.4 ± 0.2	13.1 ± 0.2	12.8 ± 0.2	13.0 ± 0.1	12.9 ± 0.1	12.9 ± 0.1
Day 23	15.2 ± 0.2	15.1 ± 0.1	15.0 ± 0.2	14.9 ± 0.1	15.1 ± 0.1	15.1 ± 0.2
Week 14	15.1 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	14.7 ± 0.1*	14.8 ± 0.1
Erythrocytes (10 ⁶ /μL)						
Day 3	6.47 ± 0.09	6.29 ± 0.09	6.23 ± 0.09	6.28 ± 0.09	6.33 ± 0.09	6.32 ± 0.09
Day 23	7.87 ± 0.13	7.68 ± 0.10	7.56 ± 0.10	7.48 ± 0.10	7.53 ± 0.08	7.67 ± 0.10
Week 14	8.20 ± 0.05	8.19 ± 0.08	8.11 ± 0.06	7.98 ± 0.09*	7.95 ± 0.06*	8.05 ± 0.06*
Reticulocytes (10 ⁶ /μL)						
Day 3	0.25 ± 0.04	0.32 ± 0.03	0.35 ± 0.02*	0.41 ± 0.03**	0.43 ± 0.04**	0.42 ± 0.04**
Day 23	0.20 ± 0.02	0.24 ± 0.03	0.30 ± 0.02*	0.27 ± 0.02	0.23 ± 0.03	0.26 ± 0.02
Week 14	0.18 ± 0.02	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.02	0.18 ± 0.02
Nucleated erythrocytes/100 leukocytes						
Day 3	1.00 ± 0.33	1.40 ± 0.40	2.20 ± 0.39*	2.50 ± 0.54*	2.30 ± 0.37*	2.80 ± 0.33**
Day 23	0.10 ± 0.10	0.20 ± 0.13	0.20 ± 0.13	0.30 ± 0.21	0.10 ± 0.10	0.80 ± 0.29*
Week 14	0.30 ± 0.15	0.30 ± 0.15	0.50 ± 0.17	0.30 ± 0.15	0.10 ± 0.10	0.44 ± 0.34
Mean cell volume (fL)						
Day 3	64.0 ± 0.4	63.8 ± 0.3	64.7 ± 0.3	64.9 ± 0.4	64.4 ± 0.5	63.1 ± 0.7
Day 23	61.0 ± 0.5	62.5 ± 0.3	62.7 ± 0.4	62.6 ± 0.4	62.3 ± 0.4	63.7 ± 0.4**
Week 14	55.6 ± 0.2	55.3 ± 0.2	55.4 ± 0.2	55.4 ± 0.2	55.5 ± 0.2	55.6 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	20.7 ± 0.2	20.9 ± 0.2	20.5 ± 0.2	20.7 ± 0.2	20.4 ± 0.3	20.5 ± 0.3
Day 23	19.3 ± 0.2	19.7 ± 0.1	19.9 ± 0.2	19.9 ± 0.2	20.0 ± 0.2	19.7 ± 0.1
Week 14	18.5 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.5 ± 0.1	18.5 ± 0.1	18.5 ± 0.2
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.5 ± 0.4	32.8 ± 0.2	31.6 ± 0.3	31.9 ± 0.4	31.7 ± 0.4	32.4 ± 0.2
Day 23	31.7 ± 0.2	31.5 ± 0.1	31.8 ± 0.2	31.9 ± 0.2	32.1 ± 0.2	30.9 ± 0.2
Week 14	33.3 ± 0.2	33.0 ± 0.2	33.3 ± 0.2	33.3 ± 0.2	33.4 ± 0.3	33.2 ± 0.2
Platelets (10 ³ /μL)						
Day 3	868.3 ± 23.8	859.6 ± 15.7	941.7 ± 28.7	924.3 ± 34.4	951.3 ± 24.1*	958.2 ± 32.1*
Day 23	857.7 ± 19.8	858.7 ± 22.0	924.7 ± 30.8	880.3 ± 32.6	996.8 ± 34.9**	1,075.7 ± 40.9**
Week 14	671.3 ± 8.4	677.6 ± 13.9	644.8 ± 10.1	679.0 ± 14.1	676.7 ± 7.6	757.6 ± 10.5**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Male (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	9
Leukocytes (10 ³ /μL)						
Day 3	7.74 ± 0.35	8.07 ± 0.24	8.24 ± 0.26	7.95 ± 0.30	8.00 ± 0.33	5.68 ± 0.58
Day 23	7.64 ± 0.44	8.98 ± 0.36	8.30 ± 0.38	8.88 ± 0.37*	9.68 ± 0.28**	9.76 ± 0.30**
Week 14	5.54 ± 0.45	5.35 ± 0.40	6.07 ± 0.60	6.21 ± 0.41	7.91 ± 0.39**	6.13 ± 0.58*
Segmented neutrophils (10 ³ /μL)						
Day 3	0.80 ± 0.07	0.87 ± 0.10	1.02 ± 0.08	1.00 ± 0.12	1.00 ± 0.05	0.72 ± 0.09
Day 23	0.78 ± 0.12	0.71 ± 0.08	0.90 ± 0.12	1.02 ± 0.08	1.29 ± 0.44	0.98 ± 0.08
Week 14	0.95 ± 0.08	0.87 ± 0.14	1.05 ± 0.09	0.93 ± 0.09	1.12 ± 0.14	0.97 ± 0.09
Bands (10 ³ /μL)						
Day 3	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10 ³ /μL)						
Day 3	6.68 ± 0.34	6.93 ± 0.22	6.96 ± 0.23	6.69 ± 0.23	6.85 ± 0.33	4.82 ± 0.49*
Day 23	6.64 ± 0.45	8.08 ± 0.34	7.28 ± 0.29	7.67 ± 0.29	8.18 ± 0.39**	8.50 ± 0.31**
Week 14	4.48 ± 0.40	4.35 ± 0.27	4.88 ± 0.52	5.13 ± 0.36	6.67 ± 0.38**	5.07 ± 0.55*
Monocytes (10 ³ /μL)						
Day 3	0.22 ± 0.05	0.21 ± 0.04	0.19 ± 0.04	0.21 ± 0.04	0.12 ± 0.03	0.09 ± 0.02*
Day 23	0.21 ± 0.06	0.19 ± 0.05	0.10 ± 0.05	0.17 ± 0.06	0.13 ± 0.05	0.22 ± 0.05
Week 14	0.07 ± 0.01	0.11 ± 0.02	0.09 ± 0.02	0.05 ± 0.02	0.10 ± 0.02	0.05 ± 0.01
Basophils (10 ³ /μL)						
Day 3	0.015 ± 0.015	0.007 ± 0.007	0.025 ± 0.013	0.006 ± 0.006	0.000 ± 0.000	0.005 ± 0.005
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.011 ± 0.011
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10 ³ /μL)						
Day 3	0.02 ± 0.01	0.07 ± 0.03	0.03 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
Day 23	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.07 ± 0.03	0.05 ± 0.02
Week 14	0.04 ± 0.03	0.03 ± 0.01	0.05 ± 0.02	0.11 ± 0.03*	0.03 ± 0.01	0.04 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	9.3 ± 0.8	7.5 ± 0.3	7.2 ± 0.2	6.7 ± 0.4	8.4 ± 0.4	12.5 ± 0.6
Day 23	10.0 ± 0.7	10.2 ± 0.5	9.4 ± 0.3	8.6 ± 0.6	10.1 ± 0.3	13.6 ± 0.7*
Week 14	13.9 ± 0.4	13.5 ± 0.4	14.7 ± 0.3	14.0 ± 0.3	14.3 ± 0.4	14.2 ± 0.3
Creatinine (mg/dL)						
Day 3	0.71 ± 0.02	0.73 ± 0.02	0.70 ± 0.02	0.71 ± 0.01	0.72 ± 0.01	0.69 ± 0.01
Day 23	0.72 ± 0.01	0.74 ± 0.02	0.73 ± 0.02	0.71 ± 0.01	0.71 ± 0.01	0.74 ± 0.02
Week 14	1.05 ± 0.03	1.08 ± 0.04	1.05 ± 0.02	1.11 ± 0.03	1.11 ± 0.03	1.06 ± 0.03
Total protein (g/dL)						
Day 3	5.6 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1
Day 23	6.0 ± 0.0	6.1 ± 0.1	6.1 ± 0.0	6.1 ± 0.0	6.2 ± 0.1	6.4 ± 0.1**
Week 14	6.7 ± 0.1	6.8 ± 0.1	6.7 ± 0.0	6.8 ± 0.1	6.8 ± 0.2	7.0 ± 0.0**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Albumin (g/dL)						
Day 3	4.0 ± 0.1	3.8 ± 0.1	3.6 ± 0.1*	3.6 ± 0.1**	3.8 ± 0.1	3.8 ± 0.1
Day 23	3.8 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.0
Week 14	3.8 ± 0.1	3.9 ± 0.0	3.8 ± 0.0	3.9 ± 0.0	4.1 ± 0.1**	4.2 ± 0.0**
Globulin (g/dL)						
Day 3	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Day 23	2.2 ± 0.1	2.4 ± 0.1	2.3 ± 0.0	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
Week 14	2.9 ± 0.0	2.8 ± 0.1	2.9 ± 0.0	3.0 ± 0.1	2.7 ± 0.2	2.9 ± 0.0
Albumin/globulin ratio						
Day 3	2.5 ± 0.2	2.4 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.5 ± 0.1
Day 23	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1
Week 14	1.3 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.6 ± 0.2**	1.5 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 3	64 ± 2	61 ± 1	61 ± 1	64 ± 2	60 ± 2	56 ± 1**
Day 23	44 ± 1	39 ± 1*	39 ± 1**	38 ± 1**	35 ± 1**	35 ± 0**
Week 14	113 ± 6	113 ± 11	110 ± 12	70 ± 4**	61 ± 3**	50 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	733 ± 14	759 ± 16	779 ± 14	794 ± 24	771 ± 24	674 ± 15
Day 23	496 ± 15	490 ± 11	506 ± 15	485 ± 15	456 ± 27	470 ± 12
Week 14	309 ± 7	294 ± 4	293 ± 10	283 ± 9*	275 ± 7**	250 ± 6**
Creatine kinase (IU/L)						
Day 3	376 ± 21	422 ± 50	476 ± 75	619 ± 96 ^b	470 ± 56	436 ± 62
Day 23	301 ± 29	273 ± 36	210 ± 25*	231 ± 26 ^b	220 ± 15	278 ± 29
Week 14	126 ± 20	125 ± 21	144 ± 20	129 ± 20	179 ± 13	118 ± 13
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 1	12 ± 1	12 ± 1	11 ± 1	12 ± 0	11 ± 1
Day 23	13 ± 0	13 ± 0	13 ± 0	12 ± 1	12 ± 0	12 ± 0
Week 14	26 ± 1	24 ± 2	22 ± 1	20 ± 1**	17 ± 1**	17 ± 1**
Bile acids (μmol/L)						
Day 3	33.8 ± 1.1	39.9 ± 0.9**	47.1 ± 0.7**	50.9 ± 1.7**	50.4 ± 2.0**	55.6 ± 4.1**
Day 23	32.1 ± 2.9	33.2 ± 1.4	37.7 ± 1.4*	41.1 ± 1.0**	43.4 ± 1.7**	44.2 ± 1.4**
Week 14	28.6 ± 1.7	31.2 ± 1.3	32.7 ± 1.5	30.9 ± 0.9	32.4 ± 0.9*	35.9 ± 2.2**
Female						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 3	45.5 ± 0.5	45.1 ± 0.4	45.3 ± 0.7	44.4 ± 0.7	44.0 ± 0.5	44.3 ± 0.5
Day 23	48.4 ± 0.4	48.9 ± 0.6	48.1 ± 0.3	48.6 ± 0.7	48.4 ± 0.5	48.5 ± 0.6
Week 14	42.1 ± 0.5	41.8 ± 0.4	42.0 ± 0.5	42.4 ± 0.5	42.5 ± 0.4	43.3 ± 0.4
Packed cell volume (mL/dL)						
Day 3	43.3 ± 0.5	43.3 ± 0.5	43.2 ± 0.6	42.3 ± 0.7	41.7 ± 0.5	42.5 ± 0.5
Day 23	46.6 ± 0.6	48.3 ± 0.7	45.9 ± 0.4	47.6 ± 0.6	47.6 ± 0.7	47.9 ± 0.7
Week 14	41.9 ± 0.3	41.9 ± 0.4	41.0 ± 0.4	42.5 ± 0.5	42.6 ± 0.4	43.3 ± 0.3*

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Female (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Hemoglobin (g/dL)						
Day 3	13.9 ± 0.2	13.8 ± 0.2	13.9 ± 0.2	13.5 ± 0.3	13.3 ± 0.1	13.7 ± 0.1
Day 23	15.5 ± 0.2	15.7 ± 0.2	15.4 ± 0.1	15.5 ± 0.2	15.6 ± 0.2	15.6 ± 0.2
Week 14	14.4 ± 0.1	14.4 ± 0.1	14.3 ± 0.1	14.5 ± 0.1	14.5 ± 0.1	14.6 ± 0.1
Erythrocytes (10 ⁶ /μL)						
Day 3	6.65 ± 0.09	6.67 ± 0.07	6.70 ± 0.10	6.52 ± 0.13	6.50 ± 0.08	6.62 ± 0.10
Day 23	7.42 ± 0.11	7.75 ± 0.13	7.35 ± 0.08	7.58 ± 0.12	7.65 ± 0.14	7.71 ± 0.15
Week 14	6.96 ± 0.03	6.99 ± 0.07	6.83 ± 0.07	7.07 ± 0.08	7.07 ± 0.07	7.17 ± 0.04
Reticulocytes (10 ⁶ /μL)						
Day 3	0.33 ± 0.03	0.34 ± 0.03	0.33 ± 0.03	0.27 ± 0.02	0.38 ± 0.02	0.28 ± 0.03
Day 23	0.14 ± 0.01	0.14 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.17 ± 0.02
Week 14	0.15 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.12 ± 0.01
Nucleated erythrocytes/100 leukocytes						
Day 3	0.40 ± 0.22	1.00 ± 0.21	0.20 ± 0.13	0.60 ± 0.16	0.70 ± 0.34	0.70 ± 0.26
Day 23	0.30 ± 0.15	0.20 ± 0.20	0.20 ± 0.13	0.30 ± 0.21	0.10 ± 0.10	0.30 ± 0.15
Week 14	0.70 ± 0.26	1.30 ± 0.40	0.40 ± 0.22	0.50 ± 0.22	0.30 ± 0.21	0.60 ± 0.22
Mean cell volume (fL)						
Day 3	65.3 ± 0.3	64.9 ± 0.4	64.4 ± 0.4	64.9 ± 0.3	64.3 ± 0.4	64.4 ± 0.3
Day 23	62.8 ± 0.3	62.4 ± 0.4	62.5 ± 0.4	62.7 ± 0.3	62.4 ± 0.5	62.2 ± 0.3
Week 14	60.2 ± 0.3	60.1 ± 0.2	60.0 ± 0.2	60.1 ± 0.3	60.3 ± 0.3	60.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	20.9 ± 0.1	20.7 ± 0.2	20.8 ± 0.1	20.7 ± 0.2	20.6 ± 0.1	20.7 ± 0.2
Day 23	20.9 ± 0.2	20.2 ± 0.2	20.9 ± 0.3	20.5 ± 0.2	20.4 ± 0.3	20.2 ± 0.3
Week 14	20.7 ± 0.1	20.5 ± 0.2	20.9 ± 0.2	20.5 ± 0.2	20.5 ± 0.2	20.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.2 ± 0.1	31.8 ± 0.4	32.2 ± 0.1	32.0 ± 0.2	32.0 ± 0.2	32.3 ± 0.2
Day 23	33.3 ± 0.3	32.4 ± 0.2	33.4 ± 0.3	32.6 ± 0.3	32.8 ± 0.3	32.5 ± 0.4
Week 14	34.4 ± 0.2	34.2 ± 0.3	34.8 ± 0.3	34.1 ± 0.3	33.9 ± 0.3	33.8 ± 0.3
Platelets (10 ³ /μL)						
Day 3	964.0 ± 24.6	860.5 ± 34.5	922.1 ± 46.2	907.5 ± 26.0	787.4 ± 26.9**	904.3 ± 41.7
Day 23	1,026.6 ± 35.1	940.7 ± 31.3 ^b	996.6 ± 37.6	963.8 ± 24.0	990.8 ± 51.8	1,042.0 ± 30.1
Week 14	620.3 ± 10.6	605.8 ± 7.7	615.8 ± 12.9	618.3 ± 14.2	640.3 ± 13.9	677.7 ± 8.5**
Leukocytes (10 ³ /μL)						
Day 3	10.24 ± 0.46	9.72 ± 0.53	9.77 ± 0.53	8.57 ± 0.40*	8.79 ± 0.41*	8.38 ± 0.67*
Day 23	7.99 ± 0.47	9.53 ± 0.57	8.78 ± 0.54	8.45 ± 0.51	9.96 ± 0.52*	10.60 ± 0.57**
Week 14	4.12 ± 0.46	2.93 ± 0.28	3.18 ± 0.27	3.57 ± 0.40	4.38 ± 0.70	4.60 ± 0.61
Segmented neutrophils (10 ³ /μL)						
Day 3	0.98 ± 0.07	1.13 ± 0.14	0.93 ± 0.09	0.84 ± 0.11	1.09 ± 0.10	1.09 ± 0.15
Day 23	0.79 ± 0.07	0.83 ± 0.12	0.84 ± 0.14	0.78 ± 0.09	0.94 ± 0.19	0.83 ± 0.09
Week 14	0.67 ± 0.11	0.48 ± 0.07	0.52 ± 0.04	0.62 ± 0.10	0.77 ± 0.29	0.73 ± 0.12
Bands (10 ³ /μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Female (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Lymphocytes ($10^3/\mu\text{L}$)						
Day 3	8.99 \pm 0.46	8.30 \pm 0.54	8.55 \pm 0.48	7.55 \pm 0.38	7.59 \pm 0.40	7.06 \pm 0.54**
Day 23	7.03 \pm 0.51	8.54 \pm 0.51	7.83 \pm 0.57	7.52 \pm 0.52	8.82 \pm 0.44*	9.51 \pm 0.52**
Week 14	3.39 \pm 0.38	2.43 \pm 0.23	2.62 \pm 0.25	2.89 \pm 0.30	3.57 \pm 0.47	3.83 \pm 0.50
Monocytes ($10^3/\mu\text{L}$)						
Day 3	0.21 \pm 0.04	0.22 \pm 0.07	0.25 \pm 0.06	0.13 \pm 0.04	0.05 \pm 0.01**	0.20 \pm 0.04
Day 23	0.11 \pm 0.03	0.13 \pm 0.03	0.05 \pm 0.03	0.10 \pm 0.04	0.17 \pm 0.05	0.17 \pm 0.04
Week 14	0.02 \pm 0.01	0.01 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.01	0.01 \pm 0.01	0.00 \pm 0.00
Basophils ($10^3/\mu\text{L}$)						
Day 3	0.000 \pm 0.000	0.000 \pm 0.000	0.018 \pm 0.012	0.000 \pm 0.000	0.018 \pm 0.012	0.008 \pm 0.008
Day 23	0.010 \pm 0.010	0.000 \pm 0.000	0.017 \pm 0.017	0.000 \pm 0.000	0.000 \pm 0.000	0.009 \pm 0.009
Week 14	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000
Eosinophils ($10^3/\mu\text{L}$)						
Day 3	0.06 \pm 0.03	0.08 \pm 0.02	0.04 \pm 0.03	0.06 \pm 0.03	0.05 \pm 0.02	0.03 \pm 0.01
Day 23	0.05 \pm 0.02	0.03 \pm 0.01	0.05 \pm 0.02	0.05 \pm 0.02	0.03 \pm 0.02	0.09 \pm 0.03
Week 14	0.04 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01	0.03 \pm 0.02	0.02 \pm 0.01	0.04 \pm 0.02
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	9.0 \pm 0.5	8.4 \pm 0.5	7.7 \pm 0.3	7.8 \pm 0.5	8.8 \pm 0.4	10.5 \pm 0.5
Day 23	12.9 \pm 0.6	12.7 \pm 0.7	12.3 \pm 0.5	12.1 \pm 0.3	11.7 \pm 0.5	11.2 \pm 0.5
Week 14	14.4 \pm 0.7	14.5 \pm 0.3	14.6 \pm 0.5	15.1 \pm 0.4	14.4 \pm 0.5	13.5 \pm 0.6
Creatinine (mg/dL)						
Day 3	0.69 \pm 0.02	0.69 \pm 0.02	0.64 \pm 0.02	0.62 \pm 0.01**	0.65 \pm 0.02*	0.62 \pm 0.01**
Day 23	0.72 \pm 0.01	0.71 \pm 0.01	0.70 \pm 0.00	0.72 \pm 0.01	0.73 \pm 0.02	0.71 \pm 0.01
Week 14	0.97 \pm 0.02	1.05 \pm 0.02	1.01 \pm 0.02	1.07 \pm 0.02*	1.03 \pm 0.03	1.04 \pm 0.03
Total protein (g/dL)						
Day 3	5.7 \pm 0.1	5.7 \pm 0.1	5.6 \pm 0.0	5.5 \pm 0.1	5.6 \pm 0.1	5.7 \pm 0.0
Day 23	6.1 \pm 0.1	6.1 \pm 0.0	6.0 \pm 0.0	6.1 \pm 0.1	6.2 \pm 0.1	6.2 \pm 0.1
Week 14	6.6 \pm 0.1	6.7 \pm 0.1	6.9 \pm 0.1	6.9 \pm 0.1	6.9 \pm 0.1*	7.1 \pm 0.1**
Albumin (g/dL)						
Day 3	3.6 \pm 0.1	3.6 \pm 0.1	3.6 \pm 0.1	3.5 \pm 0.1	3.6 \pm 0.0	3.7 \pm 0.1
Day 23	3.8 \pm 0.0	3.8 \pm 0.0	3.7 \pm 0.0	3.9 \pm 0.1	3.8 \pm 0.1	3.8 \pm 0.1
Week 14	4.2 \pm 0.1	4.0 \pm 0.1	4.3 \pm 0.1	4.2 \pm 0.1	4.3 \pm 0.1	4.6 \pm 0.1**
Globulin (g/dL)						
Day 3	2.1 \pm 0.0	2.1 \pm 0.0	2.0 \pm 0.0	2.1 \pm 0.1	2.0 \pm 0.1	2.0 \pm 0.1
Day 23	2.4 \pm 0.1	2.4 \pm 0.0	2.3 \pm 0.0	2.2 \pm 0.0*	2.4 \pm 0.1	2.4 \pm 0.0
Week 14	2.5 \pm 0.1	2.7 \pm 0.1	2.5 \pm 0.1	2.7 \pm 0.1	2.6 \pm 0.1	2.4 \pm 0.1
Albumin/globulin ratio						
Day 3	1.7 \pm 0.1	1.8 \pm 0.1	1.8 \pm 0.1	1.7 \pm 0.1	1.8 \pm 0.1	1.9 \pm 0.1
Day 23	1.6 \pm 0.0	1.6 \pm 0.0	1.6 \pm 0.0	1.8 \pm 0.0	1.6 \pm 0.1	1.6 \pm 0.1
Week 14	1.7 \pm 0.1	1.5 \pm 0.1	1.7 \pm 0.1	1.6 \pm 0.1	1.7 \pm 0.1	2.0 \pm 0.1
Alanine aminotransferase (IU/L)						
Day 3	50 \pm 1	51 \pm 2	48 \pm 2	48 \pm 2	47 \pm 2	42 \pm 2**
Day 23	37 \pm 1	37 \pm 1	34 \pm 1	35 \pm 1	34 \pm 1	33 \pm 1
Week 14	80 \pm 8	64 \pm 6	63 \pm 4	53 \pm 4**	53 \pm 3**	33 \pm 1**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Female (continued)						
n	10	10	10	10	10	10
Clinical Chemistry (continued)						
Alkaline phosphatase (IU/L)						
Day 3	642 ± 18	636 ± 23	618 ± 19	608 ± 12	585 ± 15*	474 ± 10**
Day 23	372 ± 6	388 ± 11	370 ± 8	362 ± 9	348 ± 7*	319 ± 7**
Week 14	272 ± 9	275 ± 8	262 ± 12	267 ± 7	243 ± 7*	207 ± 6**
Creatine kinase (IU/L)						
Day 3	303 ± 26	291 ± 28 ^b	326 ± 26	314 ± 34 ^b	367 ± 25	320 ± 49
Day 23	242 ± 28	233 ± 27	209 ± 15	189 ± 17 ^b	229 ± 24	245 ± 24
Week 14	149 ± 22	123 ± 15	120 ± 14	140 ± 17	125 ± 20	104 ± 9
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 1	13 ± 0	13 ± 0	12 ± 0	12 ± 0	12 ± 0
Day 23	12 ± 1	11 ± 0	11 ± 0	12 ± 0	11 ± 0	12 ± 0
Week 14	22 ± 1	21 ± 1	19 ± 0	19 ± 1	18 ± 1**	15 ± 1**
Bile acids (μmol/L)						
Day 3	27.2 ± 1.3	32.6 ± 1.9*	34.4 ± 1.8**	38.5 ± 1.8**	42.5 ± 0.9**	38.0 ± 1.7**
Day 23	23.0 ± 0.7	27.6 ± 1.5**	29.1 ± 0.9**	31.5 ± 1.1**	38.5 ± 2.9**	38.4 ± 3.6**
Week 14	29.7 ± 2.6	24.3 ± 1.3	26.2 ± 1.6	32.1 ± 4.3	25.7 ± 2.3	23.8 ± 0.9

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test

** $P \leq 0.01$

^a Data are given as mean ± standard error. Ratios were calculated and statistical tests were performed on unrounded data.

^b n=9

TABLE F2
Hematology Data for Mice in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Hematocrit (%)	49.5 ± 0.7	49.7 ± 0.4	49.3 ± 0.5	50.4 ± 0.4	49.9 ± 0.5	49.4 ± 0.6
Packed cell volume (mL/dL)	49.8 ± 0.6	50.2 ± 0.6	49.2 ± 0.5	50.6 ± 0.4	50.5 ± 0.3	48.7 ± 0.5
Hemoglobin (g/dL)	15.6 ± 0.2	15.8 ± 0.2	15.6 ± 0.2	15.9 ± 0.1	15.8 ± 0.1	15.5 ± 0.2
Erythrocytes (10 ⁶ /μL)	9.74 ± 0.10	9.84 ± 0.10	9.64 ± 0.08	9.91 ± 0.08	9.90 ± 0.06	9.45 ± 0.09
Reticulocytes (10 ⁶ /μL)	0.17 ± 0.02	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	51.2 ± 0.2	51.0 ± 0.2	50.8 ± 0.2	51.0 ± 0.2	51.0 ± 0.2	51.7 ± 0.2
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.1	16.0 ± 0.1	16.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.3 ± 0.2	31.6 ± 0.2	31.8 ± 0.2	31.5 ± 0.2	31.4 ± 0.1	31.9 ± 0.3
Platelets (10 ³ /μL)	724.2 ± 14.7	733.8 ± 9.0	732.1 ± 9.0	736.7 ± 10.5	760.6 ± 13.8*	765.4 ± 7.1*
Leukocytes (10 ³ /μL)	2.65 ± 0.29	2.92 ± 0.30	3.18 ± 0.43	3.38 ± 0.46	3.04 ± 0.43	3.02 ± 0.35
Segmented neutrophils (10 ³ /μL)	0.32 ± 0.05	0.34 ± 0.06	0.34 ± 0.05	0.43 ± 0.09	0.50 ± 0.21	0.41 ± 0.06
Bands (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10 ³ /μL)	2.31 ± 0.25	2.52 ± 0.26	2.82 ± 0.39	2.91 ± 0.40	2.49 ± 0.31	2.51 ± 0.31
Monocytes (10 ³ /μL)	0.00 ± 0.00	0.03 ± 0.01*	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01*
Basophils (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.002 ± 0.002	0.000 ± 0.000
Eosinophils (10 ³ /μL)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
Female						
n	10	10	10	10	10	2
Hematocrit (%)	49.2 ± 0.8	50.5 ± 0.5	50.2 ± 0.3	50.1 ± 0.5	50.6 ± 0.6	49.0 ± 1.0
Packed cell volume (mL/dL)	49.6 ± 0.7	50.7 ± 0.5	50.5 ± 0.5	50.6 ± 0.5	51.0 ± 0.6	49.3 ± 0.5
Hemoglobin (g/dL)	15.6 ± 0.2	15.9 ± 0.2	15.9 ± 0.2	16.0 ± 0.2	15.9 ± 0.2	15.6 ± 0.4
Erythrocytes (10 ⁶ /μL)	9.56 ± 0.11	9.73 ± 0.09	9.71 ± 0.09	9.74 ± 0.11	9.75 ± 0.10	9.42 ± 0.11
Reticulocytes (10 ⁶ /μL)	0.21 ± 0.02	0.18 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.24 ± 0.02	0.21 ± 0.06
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.21	0.10 ± 0.10	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
Mean cell volume (fL)	51.9 ± 0.2	52.0 ± 0.0	52.2 ± 0.1	52.0 ± 0.3	52.3 ± 0.2	52.0 ± 0.0
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.4 ± 0.1	16.3 ± 0.1	16.4 ± 0.1	16.3 ± 0.1	16.6 ± 0.2
Mean cell hemoglobin concentration (g/dL)	31.5 ± 0.1	31.4 ± 0.1	31.4 ± 0.1	31.5 ± 0.1	31.3 ± 0.2	31.6 ± 0.4
Platelets (10 ³ /μL)	715.2 ± 13.1	694.7 ± 20.6	718.4 ± 13.4 ^b	730.5 ± 10.6	754.2 ± 26.5 ^b	778.5 ± 1.5
Leukocytes (10 ³ /μL)	2.79 ± 0.41	3.07 ± 0.15	2.95 ± 0.17	3.18 ± 0.24	3.49 ± 0.24	2.35 ± 0.25
Segmented neutrophils (10 ³ /μL)	0.26 ± 0.03	0.36 ± 0.09	0.28 ± 0.05	0.30 ± 0.04	0.39 ± 0.07	0.34 ± 0.00
Bands (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10 ³ /μL)	2.50 ± 0.39	2.68 ± 0.12	2.62 ± 0.14	2.83 ± 0.21	3.02 ± 0.20	1.98 ± 0.24
Monocytes (10 ³ /μL)	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.02
Basophils (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10 ³ /μL)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.03 ± 0.01

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test

^a Data are given as mean ± standard error. Ratios were calculated and statistical tests were performed on unrounded data.

^b n=9

APPENDIX G

ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

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TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Inhalation Study of Cumene^a

2,000 ppm	Chamber				
	Control	250 ppm	500 ppm	1,000 ppm	
Male					
n	5	5	5	5	3
Necropsy body wt	146 ± 4	149 ± 4	148 ± 7	148 ± 5	102 ± 3**
Heart					
Absolute	0.612 ± 0.033	0.566 ± 0.015	0.576 ± 0.029	0.558 ± 0.022	0.457 ± 0.017**
Relative	4.208 ± 0.258	3.788 ± 0.036	3.893 ± 0.110	3.778 ± 0.046	4.471 ± 0.101
R. Kidney					
Absolute	0.568 ± 0.017	0.660 ± 0.022*	0.648 ± 0.026	0.690 ± 0.028**	0.563 ± 0.015
Relative	3.896 ± 0.071	4.413 ± 0.047**	4.380 ± 0.033**	4.672 ± 0.080**	5.526 ± 0.231**
Liver					
Absolute	6.282 ± 0.228	7.442 ± 0.329*	7.544 ± 0.481*	9.214 ± 0.411**	8.207 ± 0.247**
Relative	43.052 ± 0.696	49.706 ± 1.030**	50.775 ± 1.242**	62.329 ± 0.830**	80.343 ± 0.194**
Lung					
Absolute	1.068 ± 0.063	1.244 ± 0.110	1.088 ± 0.101	1.262 ± 0.082	1.070 ± 0.142
Relative	7.324 ± 0.403	8.344 ± 0.782	7.320 ± 0.456	8.582 ± 0.614	10.425 ± 1.121*
R. Testis					
Absolute	0.918 ± 0.012	0.913 ± 0.069	0.868 ± 0.104	0.947 ± 0.026	0.630 ± 0.089*
Relative	6.310 ± 0.171	6.081 ± 0.360	5.780 ± 0.537	6.421 ± 0.092	6.135 ± 0.716
Thymus					
Absolute	0.454 ± 0.013	0.459 ± 0.007	0.434 ± 0.023	0.452 ± 0.026	0.170 ± 0.011**
Relative	3.123 ± 0.116	3.080 ± 0.104	2.932 ± 0.064	3.061 ± 0.130	1.662 ± 0.103**
Female					
n	5	5	5	5	2
Necropsy body wt	120 ± 3	121 ± 3	125 ± 1	122 ± 3	93 ± 3**
Heart					
Absolute	0.494 ± 0.010	0.516 ± 0.031	0.510 ± 0.011	0.498 ± 0.015	0.440 ± 0.030
Relative	4.132 ± 0.137	4.245 ± 0.205	4.095 ± 0.098	4.069 ± 0.071	4.718 ± 0.178
R. Kidney					
Absolute	0.486 ± 0.011	0.572 ± 0.023**	0.594 ± 0.006**	0.582 ± 0.015**	0.550 ± 0.030
Relative	4.060 ± 0.102	4.706 ± 0.116**	4.768 ± 0.022**	4.761 ± 0.119**	5.900 ± 0.142**
Liver					
Absolute	5.004 ± 0.134	5.632 ± 0.163*	6.204 ± 0.183**	7.022 ± 0.251**	7.030 ± 0.010**
Relative	41.800 ± 1.128	46.390 ± 0.869*	49.764 ± 1.089**	57.371 ± 1.450**	75.544 ± 2.419**
Lung					
Absolute	0.896 ± 0.021	1.180 ± 0.129	0.978 ± 0.071	1.056 ± 0.140	0.790 ± 0.050
Relative	7.488 ± 0.216	9.787 ± 1.219	7.835 ± 0.505	8.592 ± 1.036	8.505 ± 0.797
Thymus					
Absolute	0.385 ± 0.017	0.399 ± 0.022	0.383 ± 0.008	0.357 ± 0.030	0.187 ± 0.007**
Relative	3.223 ± 0.164	3.301 ± 0.230	3.074 ± 0.034	2.903 ± 0.193	2.007 ± 0.014**

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data are reported for the 4,000 ppm groups due to 100% mortality.

TABLE G2

Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of Cumene^a

		Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm
1,000 ppm						
n	10	10	10	10	10	10
Male						
Necropsy body wt	312 ± 8	313 ± 6	322 ± 5	331 ± 4	314 ± 5	323 ± 5
Heart						
Absolute	0.850 ± 0.024	0.881 ± 0.019	0.888 ± 0.018	0.893 ± 0.015	0.885 ± 0.015	0.915 ± 0.015
Relative	2.726 ± 0.022	2.812 ± 0.021	2.754 ± 0.028	2.695 ± 0.030	2.820 ± 0.035	2.832 ± 0.037
R. Kidney						
Absolute	0.923 ± 0.024	0.980 ± 0.025	1.010 ± 0.031 ^b	1.059 ± 0.021**	1.070 ± 0.017**	1.152 ± 0.023**
Relative	2.962 ± 0.032	3.128 ± 0.051**	3.131 ± 0.056** ^b	3.194 ± 0.036**	3.411 ± 0.045**	3.561 ± 0.029**
Liver						
Absolute	9.518 ± 0.327	10.123 ± 0.267	10.260 ± 0.264	11.170 ± 0.302**	11.589 ± 0.282**	12.637 ± 0.288**
Relative	30.481 ± 0.348	32.279 ± 0.278*	31.792 ± 0.430*	33.660 ± 0.558**	36.895 ± 0.563**	39.068 ± 0.549**
Lung						
Absolute	1.431 ± 0.049	1.520 ± 0.044	1.523 ± 0.038	1.578 ± 0.027	1.548 ± 0.051	1.681 ± 0.081**
Relative	4.586 ± 0.078	4.851 ± 0.103	4.724 ± 0.090	4.765 ± 0.078	4.925 ± 0.116	5.210 ± 0.272*
R. Testis						
Absolute	1.349 ± 0.034	1.364 ± 0.022	1.376 ± 0.034 ^b	1.390 ± 0.015	1.343 ± 0.027	1.399 ± 0.017
Relative	4.333 ± 0.067	4.360 ± 0.069	4.279 ± 0.059 ^b	4.198 ± 0.051	4.281 ± 0.077	4.331 ± 0.054
Thymus						
Absolute	0.316 ± 0.011	0.326 ± 0.014	0.300 ± 0.020	0.337 ± 0.013	0.323 ± 0.012	0.316 ± 0.011
Relative	1.016 ± 0.038	1.042 ± 0.046	0.930 ± 0.056	1.017 ± 0.034	1.028 ± 0.028	0.978 ± 0.038
Female						
Necropsy body wt	195 ± 2	190 ± 3	194 ± 4	190 ± 3	185 ± 3	187 ± 4
Heart						
Absolute	0.646 ± 0.011	0.634 ± 0.011	0.633 ± 0.007	0.655 ± 0.016	0.625 ± 0.012	0.662 ± 0.016
Relative	3.312 ± 0.034	3.347 ± 0.065	3.248 ± 0.045	3.438 ± 0.045	3.380 ± 0.058	3.540 ± 0.058**
R. Kidney						
Absolute	0.637 ± 0.016	0.636 ± 0.010	0.649 ± 0.018	0.655 ± 0.017	0.645 ± 0.011	0.675 ± 0.011
Relative	3.263 ± 0.061	3.355 ± 0.049	3.322 ± 0.057	3.439 ± 0.057*	3.486 ± 0.044**	3.612 ± 0.040**
Liver						
Absolute	5.553 ± 0.130	5.669 ± 0.148	5.885 ± 0.204	5.959 ± 0.137	5.979 ± 0.133	6.923 ± 0.227**
Relative	28.442 ± 0.389	29.858 ± 0.458	30.094 ± 0.634*	31.289 ± 0.412**	32.286 ± 0.386**	36.958 ± 0.724**
Lung						
Absolute	1.093 ± 0.019	1.100 ± 0.030	1.169 ± 0.027	1.186 ± 0.028	1.098 ± 0.041	1.104 ± 0.023
Relative	5.606 ± 0.089	5.807 ± 0.168	6.003 ± 0.168	6.232 ± 0.107**	5.920 ± 0.163	5.908 ± 0.100
Thymus						
Absolute	0.288 ± 0.015	0.289 ± 0.007	0.280 ± 0.014	0.277 ± 0.011	0.270 ± 0.016	0.273 ± 0.013
Relative	1.476 ± 0.066	1.527 ± 0.041	1.432 ± 0.059	1.450 ± 0.043	1.453 ± 0.072	1.456 ± 0.050

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).^b n=9

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Inhalation Study of Cumene^a

1,000 ppm		Chamber Control	250 ppm	500 ppm
Male				
n	5	5	5	5
Necropsy body wt	27.0 ± 0.5	27.6 ± 0.6	26.7 ± 0.5	27.0 ± 0.9
Heart				
Absolute	0.142 ± 0.002	0.140 ± 0.008	0.132 ± 0.002	0.126 ± 0.007
Relative	5.268 ± 0.167	5.066 ± 0.282	4.946 ± 0.154	4.665 ± 0.160
R. Kidney				
Absolute	0.234 ± 0.005	0.270 ± 0.007**	0.250 ± 0.004	0.246 ± 0.007
Relative	8.677 ± 0.290	9.769 ± 0.152**	9.355 ± 0.181	9.122 ± 0.099
Liver				
Absolute	1.324 ± 0.051	1.520 ± 0.044**	1.570 ± 0.022**	1.808 ± 0.056**
Relative	48.933 ± 0.989	54.966 ± 0.752**	58.763 ± 1.077**	67.040 ± 0.739**
Lung				
Absolute	0.200 ± 0.014	0.268 ± 0.026*	0.198 ± 0.005	0.206 ± 0.019
Relative	7.394 ± 0.489	9.749 ± 1.067	7.412 ± 0.213	7.640 ± 0.651
R. Testis				
Absolute	0.099 ± 0.005	0.103 ± 0.003	0.102 ± 0.002	0.103 ± 0.004
Relative	3.657 ± 0.223	3.741 ± 0.104	3.834 ± 0.114	3.823 ± 0.209
Thymus				
Absolute	0.059 ± 0.003	0.051 ± 0.003	0.056 ± 0.001	0.044 ± 0.004**
Relative	2.201 ± 0.147	1.845 ± 0.132	2.107 ± 0.087	1.643 ± 0.109*
Female				
n	5	5	5	1
Necropsy body wt	22.5 ± 0.3	22.6 ± 0.7	23.3 ± 0.7	23.8
Heart				
Absolute	0.130 ± 0.003	0.126 ± 0.005	0.122 ± 0.007	0.130
Relative	5.768 ± 0.126	5.567 ± 0.174	5.234 ± 0.181	5.462
R. Kidney				
Absolute	0.172 ± 0.004	0.216 ± 0.027	0.188 ± 0.007	0.210
Relative	7.641 ± 0.230	9.608 ± 1.357	8.089 ± 0.263	8.824
Liver				
Absolute	1.150 ± 0.035	1.296 ± 0.057	1.458 ± 0.084**	1.560
Relative	50.985 ± 0.981	57.172 ± 1.306*	62.501 ± 2.211**	65.546
Lung				
Absolute	0.194 ± 0.022	0.216 ± 0.017	0.216 ± 0.015	0.200
Relative	8.599 ± 0.965	9.543 ± 0.701	9.271 ± 0.525	8.403
Thymus				
Absolute	0.076 ± 0.006	0.073 ± 0.003	0.080 ± 0.005	0.062
Relative	3.379 ± 0.238	3.251 ± 0.192	3.423 ± 0.190	2.605

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data is reported for the 2,000 and 4,000 ppm groups due to 100% mortality.

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of Cumene^a

1,000 ppm		Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	38.3 ± 0.7	37.7 ± 0.9	37.0 ± 0.8	36.1 ± 0.8	35.8 ± 0.9*	34.7 ± 0.6**
Heart						
Absolute	0.166 ± 0.003	0.167 ± 0.006	0.175 ± 0.005	0.162 ± 0.004	0.164 ± 0.006	0.152 ± 0.005
Relative	4.344 ± 0.075	4.437 ± 0.128	4.745 ± 0.136	4.501 ± 0.119	4.591 ± 0.135	4.387 ± 0.125
R. Kidney						
Absolute	0.333 ± 0.007	0.332 ± 0.007	0.337 ± 0.007	0.305 ± 0.010*	0.311 ± 0.006*	0.285 ± 0.007**
Relative	8.707 ± 0.119	8.828 ± 0.154	9.140 ± 0.204	8.460 ± 0.234	8.712 ± 0.144	8.229 ± 0.184
Liver						
Absolute	1.531 ± 0.027	1.601 ± 0.049	1.607 ± 0.041	1.591 ± 0.048	1.705 ± 0.048*	1.913 ± 0.070**
Relative	40.048 ± 0.559	42.490 ± 0.894	43.485 ± 0.551*	44.052 ± 0.746**	47.668 ± 0.795**	55.103 ± 1.501**
Lung						
Absolute	0.242 ± 0.006	0.237 ± 0.010	0.230 ± 0.007	0.219 ± 0.006	0.225 ± 0.010	0.214 ± 0.005*
Relative	6.327 ± 0.138	6.289 ± 0.208	6.218 ± 0.111	6.090 ± 0.196	6.280 ± 0.178	6.177 ± 0.124
R. Testis						
Absolute	0.117 ± 0.003	0.122 ± 0.001	0.125 ± 0.003	0.120 ± 0.002	0.117 ± 0.003	0.116 ± 0.002
Relative	3.058 ± 0.088	3.248 ± 0.079	3.390 ± 0.130	3.340 ± 0.083	3.293 ± 0.099	3.348 ± 0.054
Thymus						
Absolute	0.043 ± 0.002	0.041 ± 0.002	0.043 ± 0.002	0.043 ± 0.003	0.040 ± 0.003	0.039 ± 0.001
Relative	1.117 ± 0.069	1.096 ± 0.053	1.160 ± 0.062	1.182 ± 0.076	1.132 ± 0.084	1.124 ± 0.039
Female						
n	10	10	10	10	10	2
Necropsy body wt	32.4 ± 1.1	31.0 ± 1.2	31.4 ± 1.1	31.5 ± 1.1	29.8 ± 0.7	30.8 ± 1.3
Heart						
Absolute	0.140 ± 0.004	0.139 ± 0.002	0.140 ± 0.003	0.144 ± 0.003	0.144 ± 0.003	0.135 ± 0.005
Relative	4.335 ± 0.094	4.527 ± 0.154	4.484 ± 0.117	4.605 ± 0.132	4.848 ± 0.114*	4.404 ± 0.342
R. Kidney						
Absolute	0.209 ± 0.006	0.202 ± 0.004	0.210 ± 0.003	0.208 ± 0.004	0.218 ± 0.006	0.230 ± 0.000
Relative	6.469 ± 0.134	6.563 ± 0.179	6.740 ± 0.181	6.640 ± 0.133	7.329 ± 0.130**	7.492 ± 0.305**
Liver						
Absolute	1.453 ± 0.037	1.430 ± 0.047	1.495 ± 0.053	1.552 ± 0.045	1.593 ± 0.042*	1.910 ± 0.110**
Relative	45.016 ± 1.043	46.200 ± 0.778	47.622 ± 0.576*	49.380 ± 0.521**	53.510 ± 0.663**	62.071 ± 1.054**
Lung						
Absolute	0.223 ± 0.006	0.238 ± 0.009	0.235 ± 0.008	0.229 ± 0.006	0.240 ± 0.008	0.240 ± 0.000
Relative	6.909 ± 0.159	7.718 ± 0.302	7.501 ± 0.185	7.316 ± 0.230	8.079 ± 0.265**	7.818 ± 0.318
Thymus						
Absolute	0.059 ± 0.002	0.050 ± 0.003*	0.056 ± 0.002	0.057 ± 0.003	0.053 ± 0.002	0.052 ± 0.007
Relative	1.831 ± 0.038	1.592 ± 0.074	1.797 ± 0.055	1.810 ± 0.077	1.770 ± 0.072	1.669 ± 0.144

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX H

REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

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TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	312 ± 8	331 ± 4*	314 ± 5	323 ± 5
L. Cauda epididymis	0.1805 ± 0.0072	0.1877 ± 0.0050	0.1948 ± 0.0034	0.1738 ± 0.0040
L. Epididymis	0.4781 ± 0.0200	0.4754 ± 0.0072	0.4843 ± 0.0079	0.4502 ± 0.0103
L. Testis	1.4148 ± 0.0325	1.4627 ± 0.0148	1.4299 ± 0.0288	1.4546 ± 0.0201
Spermatid measurements				
Spermatid heads (10 ³ /mg testis)	127.6 ± 4.7	131.8 ± 4.1 ^b	128.8 ± 4.4	129.5 ± 5.4
Spermatid heads (10 ⁶ /testis)	166.8 ± 4.9	179.7 ± 5.6	170.1 ± 6.3	172.3 ± 8.2
Spermatid count (10 ⁶ /cauda epididymis)	100.28 ± 5.52	88.53 ± 4.55	95.54 ± 3.36	90.53 ± 2.32
Epididymal spermatozoal measurements				
Motility (%)	85.45 ± 3.10	81.28 ± 2.83	84.10 ± 2.03	87.62 ± 1.30
Concentration (10 ³ /mg cauda epididymal tissue)	562.4 ± 33.2	475.6 ± 28.7	492.4 ± 22.0	523.2 ± 18.2

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test

^a Data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

^b n=9

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10
Necropsy body wt (g)	195 ± 2	190 ± 3	185 ± 3	187 ± 4
Estrous cycle length (days)	5.06 ± 0.13 ^b	4.85 ± 0.11	4.80 ± 0.11	4.90 ± 0.07
Estrous stages (% of cycle)				
Diestrus	49.2	41.7	41.7	44.2
Proestrus	19.2	14.2	9.2	11.7
Estrus	15.8	25.8	28.3	25.0
Metestrus	15.8	18.3	20.8	19.2

^a Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). Evidence shows that exposed female groups differ significantly (Wilk's Criterion, $P \leq 0.05$) from the chamber control females in the relative length of time spent in the estrous stages. Exposed females spent more time in estrus and less time in proestrus than chamber control females.

^b Estrous cycle was longer than 12 days or unclear in one animal.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.3 ± 0.7	36.1 ± 0.8	36.3 ± 0.8	34.7 ± 0.6**
L. Cauda epididymis	0.0196 ± 0.0010	0.0190 ± 0.0007	0.0173 ± 0.0006	0.0171 ± 0.0006*
L. Epididymis	0.0497 ± 0.0013	0.0514 ± 0.0022	0.0493 ± 0.0010	0.0463 ± 0.0015
L. Testis	0.1119 ± 0.0027	0.1160 ± 0.0024	0.1116 ± 0.0026	0.1112 ± 0.0022
Spermatid measurements				
Spermatid heads (10 ³ /mg testis)	184.4 ± 7.6	191.3 ± 7.2	204.5 ± 7.7	202.2 ± 7.3
Spermatid heads (10 ⁶ /testis)	18.80 ± 0.77	19.77 ± 0.79	20.67 ± 0.70	20.77 ± 0.88
Spermatid count (10 ⁶ /cauda epididymis)	18.05 ± 0.95	17.62 ± 1.11	17.53 ± 1.04	14.70 ± 0.87*
Epididymal spermatozoal measurements				
Motility (%)	85.44 ± 1.96	82.75 ± 2.41	79.95 ± 2.13	83.65 ± 2.43
Concentration (10 ³ /mg cauda epididymal tissue)	931.4 ± 52.1	928.6 ± 46.1	1,017.4 ± 56.7	870.9 ± 61.5

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' (body weights), Dunnett's (tissue weights), or Dunn's (spermatid measurements) test

** $P \leq 0.01$

^a Data are presented as mean ± standard error. Differences in epididymal spermatozoal measurements between exposed groups and the chamber control group are not significant by Dunn's test.

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Inhalation Study of Cumene^a

	Chamber Control	125 ppm	250 ppm	500 ppm
n	10	10	10	10
Necropsy body wt (g)	32.4 ± 1.1	31.4 ± 1.1	31.5 ± 1.1	29.8 ± 0.7
Estrous cycle length (days)	3.96 ± 0.07	3.93 ± 0.08	3.88 ± 0.05	4.01 ± 0.12
Estrous stages (% of cycle)				
Diestrus	24.2	25.0	25.0	26.7
Proestrus	0.0	0.0	0.0	0.0
Estrus	51.7	51.7	52.5	53.3
Metestrus	24.2	23.3	22.5	20.0

^a Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages.

APPENDIX I

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

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CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF CUMENE

Cumene was obtained from Sunoco, Inc. (Philadelphia, PA), in one lot (200556852) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the study laboratory at Battelle Toxicology Northwest (Richland, WA), by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO), and by Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO). Reports on analyses performed in support of the cumene studies are on file at the National Institute of Environmental Health Sciences.

Lot 200556852 of the chemical, a colorless liquid with a sharp, penetrating, aromatic odor, was identified as cumene by the analytical chemistry laboratory using ^1H -nuclear magnetic resonance (NMR) spectroscopy and gas chromatography/mass spectrometry (GC/MS) and by Chemir/Polytech Laboratories, Inc., using infrared (IR) and ^1H -NMR spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1993, 1997; *NIST/EPA/NIH*, 1994) of cumene. The ^1H -NMR, GC/MS, and IR spectra are presented in Figures I1, I2, and I3, respectively.

The purity of lot 200556852 was determined by the analytical chemistry laboratory using gas chromatography (GC) by system A and by the study laboratory using GC by system B, C, or D (Table I1). In addition, Chemir/Polytech Laboratories, Inc., determined the moisture content of this lot by Karl Fischer titration and measured its purity by elemental analysis.

For lot 200556852, Karl Fischer titration indicated a water content ranging from approximately 50 to 220 ppm; elemental analyses for carbon and hydrogen were in agreement with the theoretical values for cumene. GC by system A detected no impurities greater than 0.05%, and the purity was determined to be approximately 100%. Using GC by system B, the area percent purity for the major cumene peak was 99.9%, and no peaks were detected with an area percent greater than 0.1%. The overall purity of lot 200556852 was determined to be greater than 99.9%.

To ensure stability, the bulk chemical was stored at controlled room temperature in the original shipping containers (55-gallon metal drums). Stability was monitored by the study laboratory during the 2-week, 3-month, and 2-year studies with GC by system B, C, or D. No degradation of the bulk chemical was detected.

VAPOR GENERATION AND EXPOSURE SYSTEM

A diagram of the vapor generation and delivery system used in the studies is shown in Figure I4. The design of the system was influenced by the relatively high boiling point of cumene (approximately 152° C) and the need to reach relatively high concentrations. Therefore, with the exception of individual chamber inlets, all vapor transport lines and dilution air were heated to the minimum temperature needed to move vapor to the chambers without condensation. A bulk supply of cumene was held in an 8-gallon stainless steel chemical reservoir and pumped through a preheater into the top of a heated glass column filled with glass beads to increase the surface area for evaporation. Heated nitrogen entering the column from below vaporized the chemical as it conveyed it out of the generator. Generator output was controlled by the delivery rate of the chemical metering pump.

Because the cumene vapor leaving the generator was above room temperature, it was transported to the exposure room at an elevated temperature to prevent condensation. In the distribution manifold cabinet, the vapor was mixed with additional heated air before it entered a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution air flow rate, all of which

were monitored by the exposure operator. The pressure in the distribution manifold was fixed to ensure constant flow through the manifold and into the chambers as the flow of vapor to each chamber was adjusted. Electronically actuated metering valves controlled the flow to each chamber. In addition, an exposure-shutoff valve, mounted in series with each chamber-metering valve, controlled vapor delivery to each chamber. Vapor was diverted to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. To start the exposure, the valves were opened to allow the flow of vapor to reach the chamber-metering valves and move into individual temperature-controlled delivery lines to each chamber. The vapor was then injected into the chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m³. A condensation particle counter (Model 3022A, TSI, Inc., St. Paul, MN) was used to count the particles in all chambers before and during generation to ensure that cumene vapor, and not aerosol, was produced. No particle counts greater than 200 particles/cm³ were detected.

VAPOR CONCENTRATION MONITORING

Summaries of the chamber vapor concentrations are given in Tables I2 through I4. Concentrations of cumene in the exposure chambers were monitored by an on-line gas chromatograph using system E (Table I1). Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 26 (2-year studies) minutes during each 6-hour exposure period using Hasteloy-C stream-select and gas-sampling valves (Valco Instruments Co., Houston, TX) in a separate, heated valve oven. The sample lines composing each sample loop were made from Teflon[®] tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of cumene in nitrogen supplied by a permeation tube standard generator (Kin-Tek Model 491, Kin-Tek Laboratories, Inc., La Marque, TX). The on-line gas chromatograph was calibrated prior to the start of each study, three times during the 2-week studies, and monthly during the 3-month and 2-year studies by a comparison of chamber concentration data to data from grab samples that were collected with charcoal sampling tubes (ORBO[™]-101, Supelco, Bellefonte, PA), extracted with toluene containing 1,2,4-trimethylbenzene as an internal standard, and analyzed by an off-line gas chromatograph using system F. The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standard solutions of cumene and the internal standard (1,2,4-trimethylbenzene) in toluene.

CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber vapor concentrations were determined with (all studies) and without (3-month and 2-year studies) animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. For the 2-week studies in rats and mice with animals present, T_{90} values ranged from 10 to 12 minutes, and T_{10} values ranged from 10 to 11 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 9 to 10 minutes without animals present and averaged 11 minutes with animals present; T_{10} values ranged from 9 to 10 minutes without animals present and from 10 to 12 minutes

with animals present. For rats and mice in the 2-year studies, T_{90} values ranged from 9 to 10 minutes without animals present and from 10 to 15 minutes with animals present; T_{10} values ranged from 8 to 9 minutes without animals present and from 9 to 13 minutes with animals present. A T_{90} value of 12 minutes was selected for all studies.

The uniformity of cumene vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; concentration uniformity with animals present in the chambers was measured once during the 2-week studies, once during the 3-month studies, and approximately quarterly during the 2-year studies. The vapor concentration was measured using the on-line gas chromatograph (system E, Table I1) with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. During the 2-week study and prior to the 3-month and 2-year studies, concentrations were measured at 12 chamber positions, one in front and one in back for each of the six possible animal cage unit positions per chamber. During the 3-month and 2-year studies, concentrations were measured at the regular monitoring port and from sample ports at levels where animals were present. Chamber concentration uniformity was maintained throughout the studies.

The persistence of cumene in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 4,000 ppm chambers in the 2-week studies and the 1,000 ppm chambers in the 3-month and 2-year studies, with (all studies) and without (3-month and 2-year studies) animals present in the chambers. In the 2-week studies, the concentration decreased to 1% of the target concentration within 77 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 25 minutes without animals present and within 28 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 25 (rats) and 24 (mice) minutes without animals present and within 35 (rats) and 27 (mice) minutes with animals present.

Samples of the test atmosphere from the distribution lines and low and high exposure concentration chambers were collected prior to the 3-month and 2-year studies and also at the beginning and end of one generation day during the 2-week, 3-month, and 2-year studies; the atmosphere samples were collected with sorbent tubes (ORBOTM-101, Supelco) and extracted with methylene chloride. Additional samples were collected from the generator reservoir and vapor trap, and all of the samples were analyzed using GC by system B to measure the stability and purity of cumene in the generation and delivery system. To assess whether impurities or degradation products co-eluted with cumene or the solvent, a second GC analysis of the samples was performed using a polar column capable of resolving compounds with similar boiling points and polarities (system G). The relative purity of cumene in the generator reservoir and vapor trap was measured using GC by systems C or D in conjunction with the stability and purity measurements described above by major peak comparison to the bulk test chemical.

No evidence of degradation of cumene was noted in any part of the exposure system. With the exception of one peak noted in the distribution line samples taken during the 3-month studies, no impurity peaks were resolved with an area >0.1% of the total peak area, and no additional impurities were detected with the polar GC analyses. The identity of the spurious peak was established by GC/mass spectrometry and by standard addition as 2-phenyl-2-propanol. The spectra for this peak (accounting for 0.14% and 0.10% of the total peak areas for the samples taken at the beginning and end, respectively, of the sampled generation day) closely matched a library reference spectrum for 2-phenyl-2-propanol (*NIST/EPA/NIH*, 1994) and the spectrum of a purchased standard of this chemical. The relative purity of all generator reservoir and vapor trap samples exceeded 99% compared to the bulk chemical, and these samples were 99.97% pure by area percent.

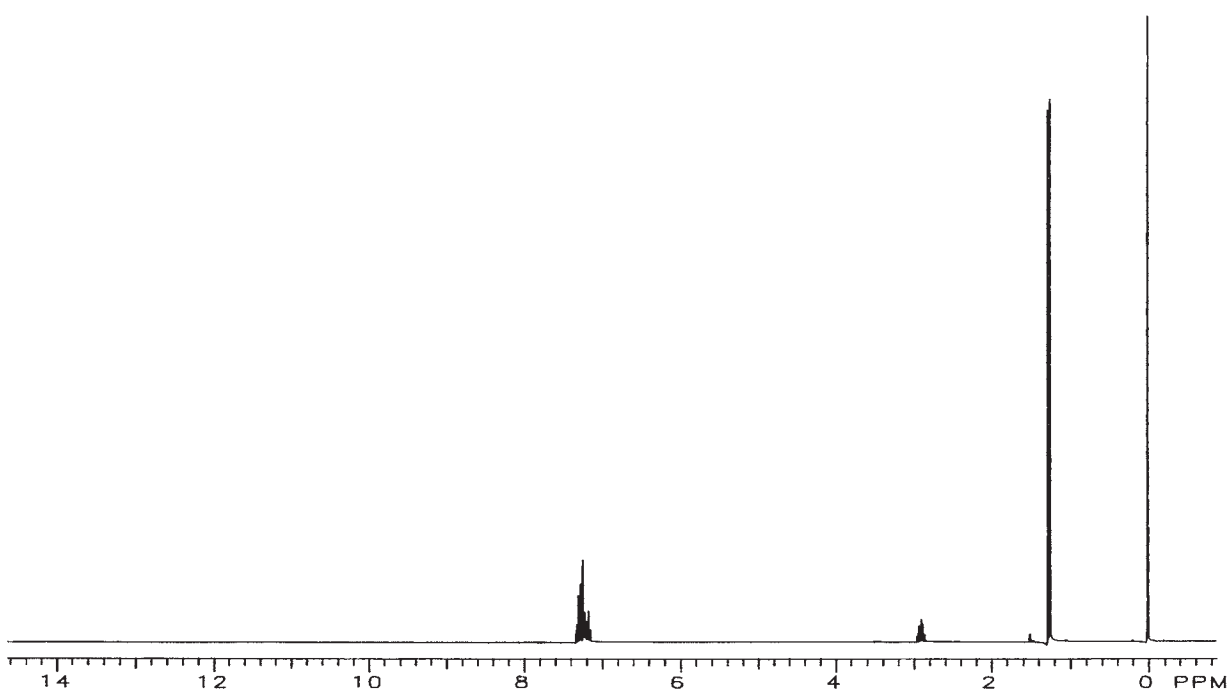


FIGURE II
 ^1H -Nuclear Magnetic Resonance Spectrum of Cumene

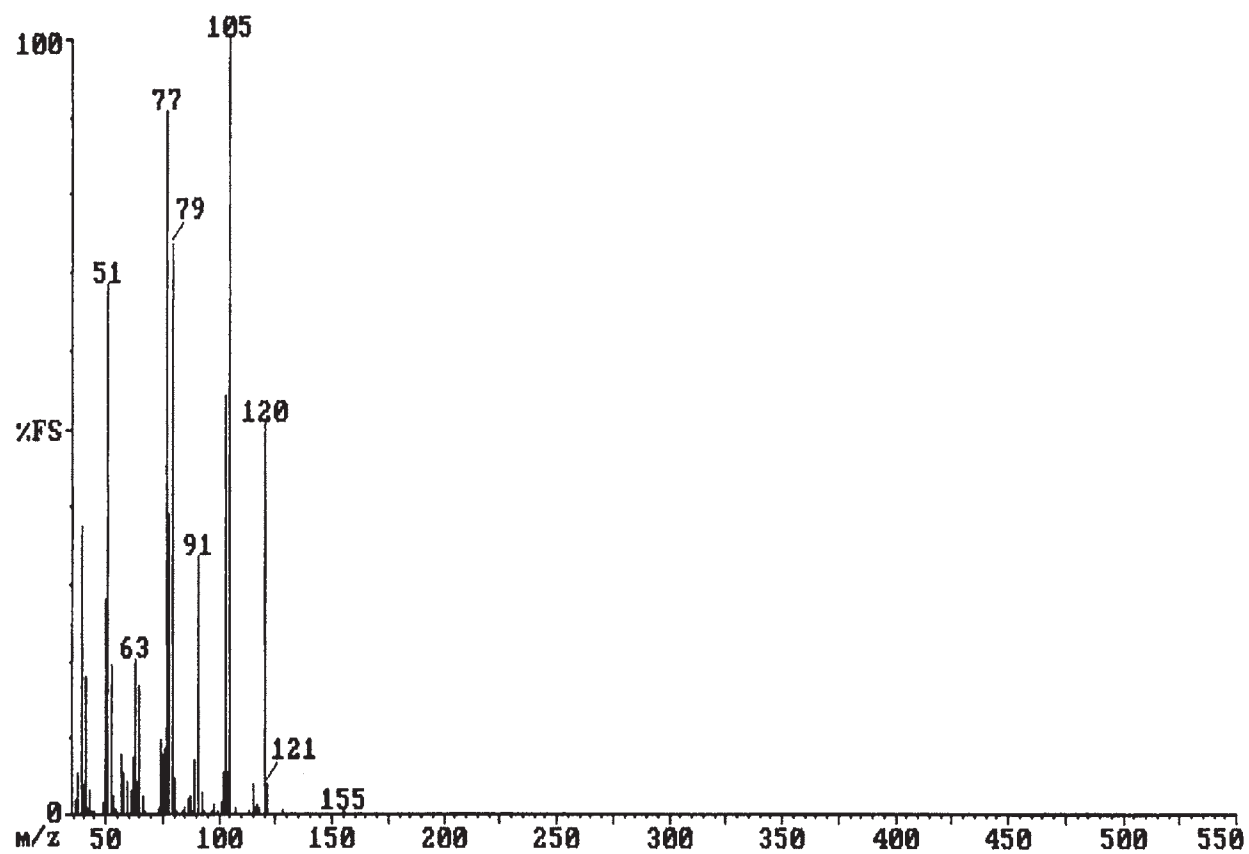


FIGURE I2
Mass Spectrum of Cumene

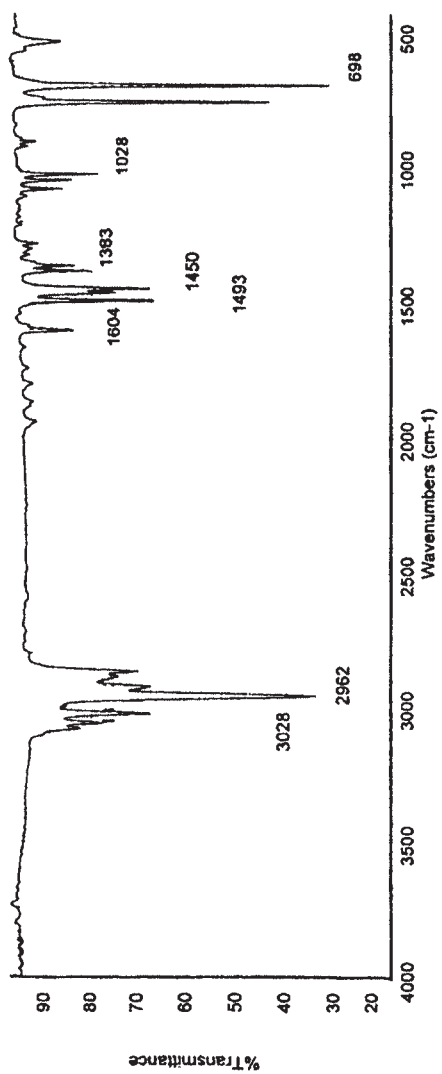


FIGURE I3
Infrared Absorption Spectrum of Cumene

TABLE II
Gas Chromatography Systems Used in the Inhalation Studies of Cumene^a

Detection System	Column	Carrier Gas	Oven Temperature Program
System A Flame ionization	DBWax, 30 m × 0.53 mm, 1.0-μm film (J&W Scientific, Folsom, CA)	Helium at 10 mL/minute	35° C for 6 minutes, then 10° C/minute to 205° C, held for 5 minutes
System B Flame ionization	Rtx-5, 30 m × 0.25 mm, 1.0-μm film (Restek, Bellefonte, PA) or DB-5, 30 m × 0.25 mm, 1.0-μm film (J&W Scientific)	Helium at 24 psi head pressure	45° C for 1 minute, then 5° C/minute to 250° C
System C Flame ionization	Rtx-5, 30 m × 0.25 mm, 1.0-μm film (Restek)	Helium at 24 psi head pressure	60° C for 1 minute, then 10° C/minute to 200° C
System D Flame ionization	Rtx-5, 30 m × 0.25 mm, 1.0-μm film (Restek)	Helium at 24 psi head pressure	80° C for 0.5 minutes, then 8° C/minute to 135° C, then 30° C/minute to 200° C
System E Flame ionization	DB-5, 15 m × 0.53 mm, 1.5-μm film (J&W Scientific)	Nitrogen at 20 mL/minute	Isothermal at 85° C
System F Flame ionization	DB-5, 30 m × 0.53 mm, 1.5-μm film (J&W Scientific)	Helium at 6 psi head pressure	90° C for 1 minute, then 16° C/minute to 150° C, held for 1 minute
System G Flame ionization	DBWax-Etr, 30 m × 0.25 mm, 0.5-μm film (J&W Scientific)	Helium at 24 psi head pressure	45° C for 1 minute, then 5° C/minute to 250° C

^a The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA).

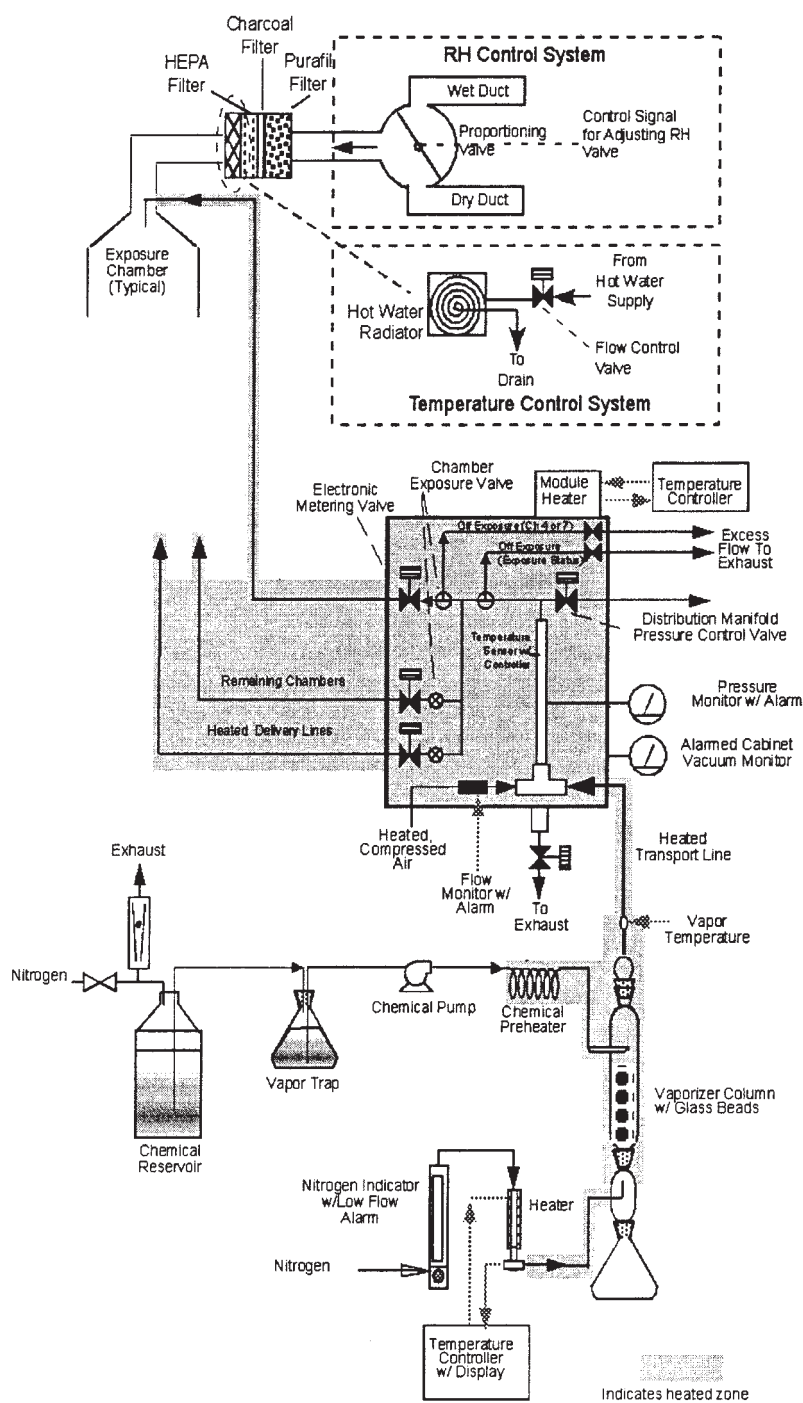


FIGURE I4
Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of Cumene

TABLE I2
Summary of Chamber Concentrations in the 2-Week Inhalation Studies of Cumene

	Target Concentration (ppm)	Total Number of Readings	Average Concentration ^a (ppm)
Rat Chambers			
	250	212	254 ± 4
	500	215	504 ± 10
	1,000	216	986 ± 30
	2,000 ^b	221	2,047 ± 65
	4,000 ^b	20	4,002 ± 58
Mouse Chambers			
	250	231	254 ± 4
	500	234	504 ± 9
	1,000 ^b	235	990 ± 31
	2,000 ^b	20	2,076 ± 49
	4,000 ^b	20	4,002 ± 58

^a Mean ± standard deviation

^b Includes data only from the first day of exposure

TABLE I3
Summary of Chamber Concentrations in the 3-Month Inhalation Studies of Cumene

	Target Concentration (ppm)	Total Number of Readings	Average Concentration ^a (ppm)
Rat Chambers			
	62.5	1,291	62.9 ± 1
	125	1,298	125 ± 3
	250	1,305	251 ± 5
	500	1,309	504 ± 10
	1,000	1,312	1,009 ± 16
Mouse Chambers			
	62.5	1,331	62.9 ± 1
	125	1,338	125 ± 3
	250	1,345	252 ± 5
	500	1,349	503 ± 10
	1,000	1,352	1,009 ± 16

^a Mean ± standard deviation

TABLE I4
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Cumene

	Target Concentration (ppm)	Total Number of Readings	Average Concentration ^a (ppm)
Rat Chambers			
	250	7,386	251 ± 5
	500	7,440	502 ± 11
	1,000	7,497	1,005 ± 23
Mouse Chambers			
	125	7,804	125 ± 2
	250	7,708	250 ± 6
	500	7,418	501 ± 13
	1,000	7,480	1,007 ± 24

^a Mean ± standard deviation

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NTP-2000 RAT AND MOUSE RATION

TABLE J1	Ingredients of NTP-2000 Rat and Mouse Ration	186
TABLE J2	Vitamins and Minerals in NTP-2000 Rat and Mouse Ration	186
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TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix ^a	0.5
Mineral premix ^b	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

^a Wheat middlings as carrier

^b Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration^a

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopherol acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
<i>d</i> -Pantothenic acid	10 mg	<i>d</i> -Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B ₁₂	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	<i>d</i> -Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

^a Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean \pm Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 \pm 0.63	13.3 – 15.7	23
Crude Fat (% by weight)	8.1 \pm 0.27	7.6 – 8.6	23
Crude Fiber (% by weight)	9.0 \pm 0.45	8.0 – 9.9	23
Ash (% by weight)	5.2 \pm 0.27	4.8 – 5.8	23
Amino Acids (% of total diet)			
Arginine	0.750 \pm 0.048	0.670 – 0.850	15
Cystine	0.225 \pm 0.025	0.150 – 0.250	15
Glycine	0.701 \pm 0.039	0.620 – 0.750	15
Histidine	0.365 \pm 0.090	0.310 – 0.680	15
Isoleucine	0.533 \pm 0.038	0.430 – 0.590	15
Leucine	1.077 \pm 0.059	0.960 – 1.150	15
Lysine	0.703 \pm 0.125	0.310 – 0.830	15
Methionine	0.402 \pm 0.049	0.260 – 0.460	15
Phenylalanine	0.615 \pm 0.035	0.540 – 0.660	15
Threonine	0.492 \pm 0.040	0.430 – 0.590	15
Tryptophan	0.135 \pm 0.018	0.110 – 0.160	15
Tyrosine	0.378 \pm 0.048	0.280 – 0.460	15
Valine	0.658 \pm 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 \pm 0.256	3.49 – 4.54	15
Linolenic	0.30 \pm 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	4,794 \pm 759	3,060 – 6,460	23
Vitamin D (IU/kg)	1,000 ^a		
α -Tocopherol (ppm)	84.2 \pm 16.60	52.0 – 110.0	15
Thiamine (ppm) ^b	7.3 \pm 1.04	5.9 – 9.2	23
Riboflavin (ppm)	6.8 \pm 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 \pm 10.50	66.4 – 98.2	15
Pantothenic Acid (ppm)	23.9 \pm 3.73	17.4 – 29.8	15
Pyridoxine (ppm) ^b	9.21 \pm 2.20	6.4 – 13.7	15
Folic Acid (ppm)	1.75 \pm 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 \pm 0.12	0.225 – 0.704	15
Vitamin B ₁₂ (ppb)	60.5 \pm 46.5	18.3 – 174.0	15
Choline (ppm) ^b	3,064 \pm 270	2,700 – 3,790	15
Minerals			
Calcium (%)	1.024 \pm 0.054	0.873 – 1.140	23
Phosphorus (%)	0.613 \pm 0.035	0.556 – 0.701	23
Potassium (%)	0.665 \pm 0.023	0.626 – 0.694	15
Chloride (%)	0.376 \pm 0.041	0.300 – 0.474	15
Sodium (%)	0.191 \pm 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 \pm 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 \pm 0.029	0.116 – 0.209	15
Iron (ppm)	182 \pm 46.7	135 – 311	15
Manganese (ppm)	54.1 \pm 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 \pm 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 \pm 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 \pm 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 \pm 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 \pm 0.074	0.20 – 0.47	14

^a From formulation

^b As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration^a

Nutrient	Mean \pm Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.32 \pm 0.151	0.17 – 0.50	23
Cadmium (ppm)	0.04 \pm 0.009	0.04 – 0.07	23
Lead (ppm)	0.07 \pm 0.026	0.05 – 0.17	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.23 \pm 0.052	0.14 – 0.36	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm) ^c	14.2 \pm 4.13	6.85 – 23.2	23
Nitrite nitrogen (ppm) ^c	<0.61		23
BHA (ppm) ^d	<1.0		23
BHT (ppm) ^d	<1.0		23
Aerobic plate count (CFU/g)	15 \pm 15	10 – 70c	23
Coliform (MPN/g)	3.1 \pm 0.2	3.0 – 3.6	23
<i>Escherichia coli</i> (MPN/g)	<10		23
<i>Salmonella</i> (MPN/g)	Negative		23
Total nitrosoamines (ppb) ^e	4.7 \pm 1.45	2.4 – 8.4	23
N-Nitrosodimethylamine (ppb) ^e	2.9 \pm 1.37	1.2 – 6.9	23
N-Nitrosopyrrolidine (ppb) ^e	1.8 \pm 0.78	0.9 – 3.1	23
Pesticides (ppm)			
α -BHC	<0.01		23
β -BHC	<0.02		23
γ -BHC	<0.01		23
δ -BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCB's	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.117 \pm 0.070	0.020 – 0.259	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.343 \pm 0.486	0.020 – 1.850	23
Endosulfan 1	<0.01		23
Endosulfan 2	<0.01		23
Endosulfane Sulfate	<0.03		23

^a All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

^b For values less than the limit of detection, the detection limit is given as the mean.

^c Sources of contamination: alfalfa, grains, and fish meal

^d Sources of contamination: soy oil and fish meal

^e All values were corrected for percent recovery.

APPENDIX K

SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female chamber control rats and mice at the end of the 2-week and 3-month studies, five male and five female sentinel rats and mice 1 week after the start of the 3-month and 2-year studies, five male and five female sentinel rats and mice at 6, 12, and 18 months in the 2-year studies, and five males and five females from the 1,000 ppm rats and 500 ppm mice at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated; fecal samples were collected from four male and five female mice. Samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test

Time of Analysis

RATS

2-Week Study

ELISA

H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
<i>Mycoplasma pulmonis</i>	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination

3-Month Study

ELISA

H-1	1 week
KRV	1 week
<i>M. arthritidis</i>	Study termination
<i>M. pulmonis</i>	1 week, study termination
PVM	1 week, study termination
RCV/SDA	1 week, study termination
Sendai	1 week, study termination

Immunofluorescence Assay

Parvovirus	Study termination
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Method and Test**Time of Analysis****RATS** (continued)**2-Year Study**

ELISA

H-1

1 week

KRV

1 week

M. arthritidis

Study termination

M. pulmonis

1 week and study termination

PVM

1 week, 6, 12, and 18 months, study termination

RCV/SDA

1 week, 6, 12, and 18 months, study termination

Sendai

1 week, 6, 12, and 18 months, study termination

Immunofluorescence Assay

Parvovirus

6, 12, and 18 months, study termination

RCV/SDA

18 months

MICE**2-Week Study**

ELISA

GDVII (mouse encephalomyelitis virus)

Study termination

MHV (mouse hepatitis virus)

Study termination

MVM (minute virus of mice)

Study termination

M. pulmonis

Study termination

PVM

Study termination

Sendai

Study termination

3-Month Study

ELISA

Ectromelia virus

Study termination

EDIM (epizootic diarrhea of infant mice)

Study termination

GDVII

1 week, study termination

LCM (lymphocytic choriomeningitis virus)

Study termination

Mouse adenoma virus-FL

Study termination

MHV

1 week, study termination

MVM

1 week

M. arthritidis

Study termination

M. pulmonis

1 week, study termination

PVM

1 week, study termination

Reovirus 3

Study termination

Sendai

1 week, study termination

Immunofluorescence Assay

GDVII

Study termination

MCMV (mouse cytomegalovirus)

Study termination

Parvovirus

Study termination

Method and Test**MICE** (continued)**2-Year Study**

ELISA

Ectromelia virus
 EDIM
 GDVII
 LCM
 Mouse adenoma virus
 MHV
 MVM
M. arthritidis
M. pulmonis
 PVM
 Reovirus 3
 Sendai

Time of Analysis

6, 12, and 18 months, study termination
 6, 12, and 18 months, study termination
 1 week, 6, 12, and 18 months, study termination
 6, 12, and 18 months, study termination
 6, 12, and 18 months, study termination
 1 week, 6, 12, and 18 months, study termination
 1 week
 Study termination
 1 week, study termination
 1 week, 6, 12, and 18 months, study termination
 6, 12, and 18 months, study termination
 1 week, 6, 12, and 18 months, study termination

Immunofluorescence Assay

EDIM
 GDVII
Helicobacter billis
Helicobacter hepatica
 LCM
 MCMV
 MHV
M. arthritidis
 Parvovirus

12 and 18 months, study termination
 6 and 12 months
 18 months
 18 months
 12 months
 Study termination
 Study termination
 Study termination
 6, 12, and 18 months, study termination

RESULTS

All results were negative.

APPENDIX L

CHARACTERIZATION OF *K-ras* AND *p53* MUTATIONS IN LUNG NEOPLASMS OF MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

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TABLE L1 <i>K-ras</i> Mutations in Lung Neoplasms of B6C3F1 Mice in the 2-Year Inhalation Study of Cumene	200
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CHARACTERIZATION OF *K-ras* AND *p53* MUTATIONS IN LUNG NEOPLASMS OF MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

INTRODUCTION

Following exposure of male and female B6C3F1 mice by inhalation to 125 (females only), 250, 500, or 1,000 (males only) ppm cumene for 2 years, there were increased incidences of lung neoplasms in all groups of exposed males and females, specifically alveolar/bronchiolar adenomas and carcinomas. Cumene was not genotoxic in several studies involving bacterial and mammalian cells in culture and in *in vivo* studies involving mice (Appendix E; USEPA, 1997; HSDB, 2003). *In vitro* cell transformation assays using BALB/3T3 mouse embryo cells and unscheduled DNA synthesis assays using rat primary hepatocytes yielded conflicting results regarding a cumene effect that were not reproducible. Cumene induced a small, but significant, increase in micronucleated erythrocytes in bone marrow of male rats following intraperitoneal injection of doses ranging from 78 to 2,500 mg/kg body weight per day for 3 days (Appendix E).

Mouse alveolar/bronchiolar adenomas, which are the most common spontaneous and chemical-induced lung tumors in mice, are similar in histomorphology and molecular characteristics, including activation of the *K-ras* gene, to human adenocarcinomas (Meuwissen and Berns, 2005). The patterns of mutations in cancer genes, such as *ras* and *p53*, have been found to aid in the understanding of tumorigenesis (Harris, 1993; Maronpot *et al.*, 1995; Osada and Takahashi, 2002; Le Calvez *et al.*, 2005). For example, in some neoplasms, the profile of activating mutations in *ras* genes or inactivating mutations in the *p53* gene are specific for particular chemicals and differ from those detected in spontaneous neoplasms (Sills *et al.*, 1999, 2004).

In the present study, 52 alveolar/bronchiolar neoplasms from B6C3F1 mice exposed to cumene for 2 years were examined for mutations in exons 1 and 2 of *K-ras*, for overexpression of mutant *p53* protein using immunohistochemistry, and for mutations in exons 5 through 8 of *p53* gene. Other studies to assess changes in the *K-ras*/MAP kinase signaling pathway are in progress.

MATERIALS AND METHODS

Lung Neoplasms

Male and female B6C3F1 mice were exposed to 0, 125 (females only), 250, 500, or 1,000 (males only) ppm cumene (50 animals per group per sex) by inhalation for 6 hours per day, 5 days per week for 2 years. At necropsy, tissues were fixed in 10% neutral-buffered formalin, routinely processed, embedded in paraffin, sectioned to a thickness of 5 μ m, and stained with hematoxylin and eosin. Subsequently, five unstained serial sections, 10 μ m thick, were prepared from paraffin blocks containing alveolar/bronchiolar adenomas or carcinomas. In order to isolate adequate amounts of DNA, neoplasms greater than 1 mm in diameter were identified for analysis. Fifty-two cumene-induced alveolar/bronchiolar neoplasms (six adenomas and 46 carcinomas), seven spontaneously occurring carcinomas, and six normal lung tissues were evaluated for *K-ras* mutations in exons 1 and 2 (codons 12, 13, and 61) and *p53* mutations in exons 5 through 8.

DNA Isolation, Amplification, and Cycle Sequencing

DNA was isolated and extracted from paraffin-embedded sections containing lung neoplasms and normal lung tissue and amplified by polymerase chain reaction (PCR). Details of the use of nested primers for *K-ras* and *p53* genes have been described previously (Sills *et al.*, 1995; Lambertini *et al.*, 2005). Positive DNA controls for *K-ras* and *p53* mutations and controls lacking DNA were run with all sets of reactions. PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Inc., Valencia, CA). The purified samples

were sequenced utilizing a cycle sequencing kit (USB Corporation, Cleveland, OH), which incorporated α -33p-dideoxynucleotide triphosphate (ddNTP) terminators (A, C, G, T) into the sequencing products. Detected mutations were confirmed by repeat analysis, starting from amplification of the original DNA extract.

Immunohistochemistry for p53 Protein

Alveolar/bronchiolar adenomas and carcinomas were examined for *p53* protein expression by immunohistochemical analysis using an avidin-biotin-peroxidase detection system [VECTASTAIN Elite ABC Kit (Rabbit IgG), Vector Laboratories, Burlingame, CA]. The immunohistochemical staining for expression of mutant *p53* protein was performed as previously described (Hong *et al.*, 2000; Sills *et al.*, 2004).

RESULTS

A higher frequency of *K-ras* mutations (45/52, 87%) was observed in the cumene-induced lung neoplasms, as compared to spontaneous lung neoplasms from control animals (historical controls, 33/117, 28%; current controls, 1/7, 14%) (Table L1). The predominant *K-ras* mutations were codon 12 G to T transversions (GTT) and codon 61 A to G transitions (CGA). These two mutations were found at frequencies of 21% (11/52) and 25% (13/52), respectively, in the neoplasms from exposed mice, compared to 0.008% (1/124) and 2% (3/124), respectively, in lung neoplasms from control mice in the historical database. Three codon 12 CGT mutations and one codon 61 CTA mutation were found in exposed groups but none in spontaneous lung neoplasms (0/124) (Table L1).

p53 mutations were identified in 52% (27/52) of the cumene-induced lung neoplasms; none were identified in seven spontaneous carcinomas, and six were identified in normal lung tissue (Table L2). The predominant *p53* mutations were identified in exon 5 (24/27, 89%) (Table L2).

There were dose-related increases in the incidences of *K-ras* and *p53* mutations; however, a similar spectrum of both mutations was detected in cumene-induced neoplasms regardless of whether the neoplasms were adenomas or carcinomas. The *p53* protein expression was detected in 56% (29/52) of the cumene-induced lung neoplasms, mostly corresponding to *p53* mutations, and was localized to the nucleus, compared to 14% (1/7) in the spontaneously occurring neoplasms without *p53* mutation.

DISCUSSION

A high frequency (87%) of *K-ras* mutations was identified in cumene-induced alveolar/bronchiolar neoplasms compared to that in spontaneous alveolar/bronchiolar neoplasms from control B6C3F1 mice (28% historical database; 14% current study). The predominant mutations were *K-ras* codon 12 G to T transversions (GGT to GTT, 21%) and codon 61 A to G transitions (CAA to CGA, 25%), which clearly differed from those identified in control mice (0.008% and 2%, respectively). Point mutations at codon 12 of the *K-ras* gene are activating mutations, rendering *ras* insensitive to the down-regulatory action of GTPase activating proteins, thereby locking the protein in the active state and promoting cellular transformation (Ellis and Clark, 2000). G to T transversions are commonly detected DNA base changes associated with active oxygen species and are consistent with 8-OH-G adducts produced during oxidative damage to DNA (Shigenaga and Ames, 1991; Tchou *et al.*, 1991; Janssen *et al.*, 1993). Exposure of B6C3F1 mice to ozone or vanadium pentoxide may have resulted in the generation of hydroxyl radicals, which could have induced G to T transversions at codon 12 of the *K-ras* gene (Sills *et al.*, 1995; Devereux *et al.*, 2002). Interestingly, G to T transversions in *K-ras* codon 12 are the most common mutations detected in human adenocarcinomas (Rodenhuis *et al.*, 1987). In human lung tumors, *K-ras* codon 12 G to T mutations appear to correlate with DNA adducts of benzo(a)pyrene and are associated with smoking (Reynolds *et al.*, 1987). It is possible that smoking in combination with cumene exposure in humans may have an additive effect on *K-ras* mutations.

The high frequency and pattern of *K-ras* mutations in mouse lung tumors may directly depend on the nature of the chemical carcinogen or its metabolites. In the present study, the development of lung neoplasms in the B6C3F1 mouse exposed to cumene may involve multiple carcinogenic processes, including direct DNA damage and/or indirect DNA damage such as oxidative stress. There were neoplasms without mutations of the *K-ras* gene, suggesting that other genetic events should be considered (Anderson *et al.*, 1992; Loeb, 2001).

Previous studies showed that benzene is carcinogenic and genotoxic (Gut *et al.*, 1996; Snyder and Hedli, 1996; Valentine *et al.*, 1996; Abernethy *et al.*, 2004). Side-chain oxidation of cumene is rapid and extensive and occurs in both hepatic and extrahepatic tissues, including the lung (Sato and Nakajima, 1987), with the secondary alcohol 2-phenyl-2-propanol being the principal metabolite in rats (RTI, 1989; USEPA, 1997) and humans (Lee, 1987; USEPA, 1997). The C-isopropyl bonds are readily cleaved, and the remaining electrophilic carbon moiety may form DNA adducts and cause subsequent DNA damage.

Interestingly, the *in vivo* tumor response in the present study did not show an exposure concentration-related response, suggesting that *K-ras* mutational analysis may be a more sensitive method for identifying dose response with cumene.

A high frequency (52%) of *p53* mutations were detected in cumene-induced alveolar/bronchiolar neoplasms that were correlated to *p53* protein expression (56%) by immunohistochemistry. The presence of *p53* protein expression without *p53* mutation could be due to *p53* mutations outside the regions exons 5 through 8 examined or possibly to alterations of other proteins downstream of *p53* (Greenblatt *et al.*, 1994). The predominance of cumene-induced alveolar/bronchiolar neoplasms containing *p53* mutations is consistent with these mutations, providing a selective advantage for unregulated growth and the avoidance of apoptosis (Greenblatt *et al.*, 1994; Harris, 1996; Osada and Takahashi, 2002; Rodin and Rodin, 2005). A study of aflatoxin-B1 (AFB1)-induced mouse lung tumors also found a high proportion (>70%) of tumors with *p53* accumulation and mutations (Tam *et al.*, 1999). In addition, 84% *p53* mutations were detected in lung tumors of mice exposed transplacentally to AZT (Hong *et al.*, 2007), while other studies have found a low frequency of *p53* mutations in methylene chloride-induced mouse lung tumors (Hegi *et al.*, 1993), and no *p53* mutation was present in vanadium pentoxide-induced mouse lung tumors (Devereux *et al.*, 2002). Unlike the mostly random mutation pattern for the AFB1-induced tumors (Tam *et al.*, 1999), the cumene-induced tumors had specific *p53* mutations. Only exon 5 (24/27, 89%) and exon 7 (11%) appeared to account for the *p53* mutations.

The data on *K-ras* and *p53* mutations provide evidence that these genetic alterations play an important role in cumene-induced mouse lung carcinogenesis. Additional evidence (Santillo *et al.*, 2001) showed that *K-ras* activation may play a major role in the formation of these neoplasms. Other microarray studies to investigate the role of MAP kinase signaling pathway in these neoplasms are in progress.

In conclusion, the patterns of mutations identified in the lung tumors suggest that DNA damage and genomic instability may be the contributing factors to the mutation profile and development of lung cancer in these mice. The molecular alterations identified in the cumene-induced lung neoplasms affect the same pathways as those reported in human lung cancer, suggesting that the response in the mouse may be of relevance to humans.

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TABLE L1
K-ras Mutations in Lung Neoplasms of B6C3F1 Mice in the 2-Year Inhalation Study of Cumene^a

Exposure Concentration (ppm)	Activate K-ras	Codon 12 (GGT)				Codon 13 (GGC)	Codon 61 (CAA)			
		(GAT)	(TGT)	(GTT)	(CGT)		(CGA)	(CAT)	(CAC)	(CTA)
Historical controls ^b	33/117 (28%)	14	5	1	0	6	2	4	1	0
Chamber controls	1/7 (14%)	0	0	0	0	0	1	0	0	0
Cumene total	45/52 (87%)	6	5	11	3	4	13	0	2	1
125	1/4 (25%)	0	1	0	0	0	0	0	0	0
250	10/13 (77%)	0	0	1	2	0	5	0	2	0
500	17/18 (94%)	4	1	6	0	2	4	0	0	0
1,000	17/17 (100%)	2	3	4	1	2	4	0	0	1

^a Only neoplasms greater than 1 mm in diameter were used; 125 ppm, females only; 1,000 ppm, males only.

^b Historical incidences of spontaneous lung neoplasms in control B6C3F1 mice (Hong *et al.*, 2007)

TABLE L2
p53 Mutations in Lung Neoplasms of B6C3F1 Mice in the 2-Year Inhalation Study of Cumene^a

Exposure Concentration (ppm)	Activate p53 ^b	IHC Positive	Exon 5	Exon 7
Chamber controls	0/7	1/7 (14%)	0	0
Cumene total	27/52 (52%)	29/52 (56%)	24	3
125	0/4	1/4 (25%)	0	0
250	5/13 (38%)	6/13 (46%)	4	1
500	11/18 (61%)	8/18 (44%)	10	1
1,000	11/17 (65%)	14/17 (82%)	10	1

^a Only neoplasms greater than 1 mm in diameter were used; 125 ppm, females only; 1,000 ppm, males only.

^b No mutation detected for p53 at exons 6 and 8 in the samples examined